Medical Library

JUL 6 1942

JOURNAL OF NEUROPHYSIOLOGY

J. NEUROPHYSIOL.

EDITORIAL BOARD

JOHN F. FULTON

R. W. GERARD R. LORENTE de NÓ

ADVISORY BOARD

E. D. Adrian (Cambridge)
Percival Bailey (Chicago)
Philip Bard (Baltimore)
G. H. Bishop (St. Louis)
F. Bremer (Brussels)
Detlev W. Bronk (Philadelphia)
Stanley Cobb (Boston)
Hallowell Davis (Boston)
U. Ebbecke (Bonn)
J. C. Eccles (Sydney)
Joseph Erlanger (St. Louis)
Wallace O. Fenn (Rochester)
Alexander Forbes (Boston)
H. S. Gasser (New York)
Ragnar Granit (Stockholm)
W. R. Hess (Zürich)
Marion Hines (Baltimore)
Davenport Hooker (Pittsburgh)
M. Ozorio de Almeida (Rio de Janiero)
Wilder Penfield (Montreal)
H. Piéron (Paris)
S. W. Ranson (Chicago)
C. S. Sherrington (Cambridge)

JULY 1942

VOL. V ISSUED BI-MONTHLY NO. 4

PUBLISHED BY CHARLES C THOMAS

220 EAST MONROE STREET, SPRINGFIELD, ILLINOIS, U.S.A.

JOURNAL OF NEUROPHYSIOLOGY

SCOPE: The aim of the *Journal of Neurophysiology* is to provide a channel for *prompt* publication of original contributions on the functions of the nervous system, peripheral and central. Materials submitted may include any phase of the subject amenable to experimental analysis; papers on pure morphology or neuropathology are not acceptable. Clinical, psychological and zoological studies will be considered only if primarily experimental in character.

MANUSCRIPTS: Manuscripts should be addressed to THE EDITORS, JOURNAL OF NEUROPHYSIOLOGY, 333 Cedar Street, New Haven, Connecticut, U.S.A. Papers should be concise and submitted in typewritten form, preferably in triple space, with tabular matter and passages for small print in double space and on separate sheets. All materials should be mailed flat. A brief summary should be included.

REFERENCES: Citations to literature should be cited by number in the text, arranged in alphabetical order and typed single space on a separate sheet. Names and initials of the authors with full title and full pagination of the paper or book must be given in each case. Names of journals should be abbreviated in accordance with the system used in *A world list of scientific periodicals published in the years 1900–33* (Oxford University Press, 1934). For details concerning form see: A style book containing suggestions for the preparation of manuscripts. *J. Neurophysiol.*, 1939, *2:* 89–99. Reprints of this may be obtained by contributors from the Editorial Office.

COSTS CHARGEABLE TO AUTHORS—*Illustrations:* The allowance for free illustrations varies with the length of the paper and the character of the illustrative matter, but not more than four 3×3.5 inch half-tones (or their equivalent in area) will be allowed free to any contributor. When a paper has been accepted for publication, authors will be notified promptly of any charges for illustrations in excess of the free allowance. These will be charged to the author at a rate of approximately $5.00 for a 3×3.5 inch (7.6×8.9 cm.) half-tone and at a somewhat lower rate for zinc cuts. All illustrations, especially photographs, should be sharp and clear and submitted ready for reproduction, *i.e.*, with lettering, mounting and arrangements of figures completed.

Special typography. Tables, mathematical and chemical formulae and also text set up in small print (8 point) in excess of 10 per cent of composition costs are chargeable to the author.

Proof corrections. Corrections in galley proof will be allowed without charge up to 5 per cent of the original cost of composition of 10 point type. The cost of changes in page proof, if made by the author, will be borne by him. *Page proof* will be submitted to authors only when special questions arise. Manuscripts and figures will be returned on request after the editorial office has corrected page proof.

Reprints. Twenty-five reprints of each paper will be supplied without charge; larger numbers can be purchased at cost; delivery will be prompt. Reprints should be ordered on forms accompanying the galley proof and returned with it to the Editorial Office.

SUBSCRIPTIONS: The subscription rate for the volume of six bi-monthly issues is $6.00 in the United States, Canada and Latin America, and $6.50 in other countries. Single copies are $1.50 postpaid. You may pay in advance to the publisher: CHARLES C THOMAS, 220 East Monroe Street, Springfield, Illinois, U.S.A.

Delivery to countries in war zones will be attempted but delivery is not guaranteed, nor is replacement guaranteed, unless request is received following a period of sixty days after the publication of any issue.

Entered as second-class matter February 13, 1939 at the postoffice at Springfield, Illinois, and Menasha, Wisconsin under the Act of March 3, 1879

COPYRIGHT, 1942, BY CHARLES C THOMAS

POSITIONAL NYSTAGMUS IN CEREBELLAR LESIONS

E. A. SPIEGEL AND N. P. SCALA

Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa.

(Received for publication February 4, 1942)

INTRODUCTION

THE EXISTENCE of nystagmus in cerebellar lesions is rather controversial. One group of authors denies a nystagmus of strictly cerebellar origin and is inclined to explain nystagmus in cerebellar diseases as due to accompanying lesions of the vestibular nuclei and fiber systems, *e.g.*, due to edema or pressure in cerebellar tumor or abscess (1, 2, 3, 4, 5); others admit that a lesion limited to the cerebellum may produce at least a transient nystagmus (6, 7, 8) particularly if it affects the deep cerebellar nuclei (9).

The differences of opinion may, in part, be due to differences in the technique of observation. In the majority of the clinical as well as of the experimental studies of cerebellar lesions no account is given whether the influence of the position of the head upon the appearance of spontaneous nystagmus was ascertained although Brun's symptom (vertigo influenced by position of the head) is not infrequent in tumors of the posterior fossa. Otologists (1, 10, 11) seem to have paid more attention to the influence of position upon various types of nystagmus than neurologists, and particularly Nylén showed that positional nystagmus may be observed rather frequently (in 80 per cent) in tumors of the posterior fossa. As pointed out by Nylén himself the nystagmus elicited by changes of position in cases of tumors may be due to changes in pressure or traction affecting the vestibular nuclei, or to changes in the blood supply or of the circulation of the spinal fluid. The question, however, is not answered by this material whether in non space-taking lesions of the cerebellum nystagmus appears more readily in certain positions.

Our attention was directed to this problem in former experiments in which lesions were placed in the posterior part of the vermis (Sven Ingvar's lobus posterior medianus) of cats and preponderantly vertical nystagmus appeared that was increased in abnormal positions, particularly in supine position of the animal (12). The question arose whether positional nystagmus may be elicited from the posterior part of the vermis only or also from other areas of the cerebellum and what mechanism is responsible for this phenomenon.

METHODS

The experiments were performed on 39 cats. The lesions were placed in the various parts of the cerebellar cortex by electrocautery and in the cerebellar nuclei by electrolysis with the aid of the Horsley-Clarke stereotaxic apparatus. The animals were operated under ether anesthesia, so that the effect of the anesthetic subsided quickly and the eventual ocular reactions could be observed within half an hour after operation. The eyes were

routinely observed while the animal was tied in normal prone position to the Harvard animal board and the head fixed with the vertex upward in a Czermak head holder which was modified in such a way that the eyes remained free. Thus the relative position of head to trunk remained fixed and the effect of neck reflexes elicited by changes in the tension of the neck muscles was excluded.

The eyes were as a rule examined in the following positions of the animal: normal, right, and left side position, and supine position. The abnormal positions were obtained by slowly tilting the board on which the animal was strapped in order to avoid eventual kinetic labyrinthine reactions. Care was also taken to support the body of the animal sufficiently in the abnormal positions so that the relationship between trunk and head remained unchanged. The extent of the lesions was studied macroscopically and in decisive cases also microscopically on serial sections stained by Weil's method and with cresyl violet.

Results

Localization. Rather small lesions of the lobus posterior medianus may be sufficient to produce positional nystagmus as shown by the following experiment.

Experiment 1. (*Cat. 8*). *Cauterization of the lobus posterior medianus, Oct. 23, 1940, 2–3 p.m. After operation the eyes deviated downward; nystagmus upward, of small amplitude, 36 jerks in 30 sec.*

Postoperative notes. First day (Oct. 24). In the *normal position* there was no nystagmus. Between the volitional eye movements there were one to 2 jerks upward appearing per minute. It was difficult to decide whether these jerks were involuntary or not.

Supine position. (Vertex downward). There was nystagmus toward the lower lid.

1st 10 sec.—25 jerks of large amplitude
2d 10 sec.—15 " " " "
3d 10 sec.—14 jerks, amplitude of nystagmus also decreased

In the *normal position* there was no nystagmus. In the *lateral position* (with the left ear downward), there was minimal rotatory nystagmus to the right. With the right ear downward, there was no nystagmus.

Second day (Oct. 25). No nystagmus was present in the *normal position*, but in the *supine position* it was present toward the lower lid.

1st 10 sec.—25 jerks 4th 10 sec.—9 jerks
2d 10 sec.—16 " 5th 10 sec.—5 "
3d 10 sec.—11 "

Fourth day (Oct. 27). In the *normal position* the animal continued to show no nystagmus. *Supine position.* There was nystagmus toward the lower lid of small amplitude.

1st 10 sec.— 5 jerks
2d 10 sec.— 5 "
3d 10 sec.— 3 "

Fifth day (Oct. 28). In *normal position* no nystagmus was present. In *supine position* 3 trials showed no definite nystagmus. In 1 trial 3 jerks of small amplitude were observed in the 1st 10 sec. only.

Sixth day (Oct. 29). In *normal position* 1–2 upward jerks appeared. In *supine position* 2 trials gave no definite reaction. In 1 trial there were in the 1st 10 sec. 1–2 rotatory ↷ jerks and 1 to the upper lid.

Eighth day (Oct. 31). In *normal position* there were 1–2 upward jerks per minute. In supine position (vertex downward) 2 trials gave no definite reaction. In 1 trial 1–2 rotatory ↷ jerks were observed. The animal was in excellent condition.

Eleventh day (Nov. 3). In *normal position* there were 1–2 jerks per minute; their direction was ↑ and slightly to the left. In supine position (vertex downward) there was nystagmus toward the lower lid, of very small amplitude.

1st 10 sec.— 4 jerks 4th 10 sec.—0 jerks
2d 10 sec.— 4 " 5th 10 sec.—0 "
3d 10 sec.— 1 "

On the 25th and 33d postoperative days there was no nystagmus observed in the normal as well as in supine position. The animal was sacrificed on the 33d day (Nov. 25).

Autopsy. Dura adherent to pyramis and uvula. Lesion of these lobules extended to the medial part of the left paramedian lobe (Fig. 1).

Histological Examination. Sections through the beginning of the medulla oblongata reveal lesion of the surface of the *pyramis cerebelli.* On more cranial sections (through the caudal third of the oblongata) in the deeper parts of the pyramis only the central white matter of the pyramis cerebelli is affected and some Purkinje cells on the ventral surface of this lobe are destroyed or show tigrolysis. In these levels the *uvula* is severely injured in all layers except the dorsal surface (Fig. 2 shows demyelination of the medullary layer of its lobules and necrosis of the granular layer). In the *left paramedian lobe* the most ventral lobule shows loss of cells in the granular and Purkinje cell layers on its ventral surface. The *right paramedian lobe* is intact. In the most anterior part of the uvula (sections through the triangular nucleus) the lesion of the uvula decreases in extent and intensity and the *nodulus* shows only slight impairment of the Purkinje cells. The *deep cerebellar nuclei* do not show a definite lesion. There is only slight increase in the number of glial nuclei in the region of the nucleus fastigii. In the Weil preparation one finds on one place a slight disintegration of the myelin sheaths dorso-lateral to the left nucleus fastigii. In the *medulla oblongata* there are only in its most caudal levels (where the central canal is still closed) small foci of necrosis in the nucleus Goll of one side and in the ncleus Burdach of both sides, and one small focus in the lateral part of the left dorsal nucleus of the vagus. The rest of the oblongata and pons including the vestibular nuclei and the posterior longitudinal fasciculi do not show any lesions. In the caudal part of the fourth ventricle necrotic material is found.

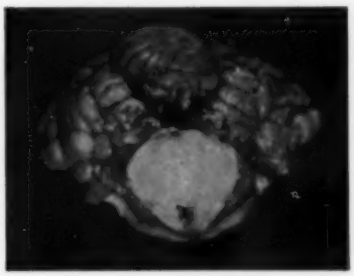

FIG. 1. Cat 8.

Summary: Maximal changes in the uvula, slight lesion on the surface of the pyramis cerebelli, and in a lobule of the left paramedian lobe. Very slight injury to the nodulus. No definite lesion of the deep cerebellar nuclei. Vestibular nuclei and their fiber systems intact.

Positional nystagmus may appear, not only following lesions of the lobus posterior medianus, but also after lesion of other parts of the vermis. While, however, rather small lesions of the lobus posterior medianus produced a definite positional nystagmus of several days duration, the nystagmus following lesions of the middle parts of the vermis between sulcus primarius and sulcus prepyramidalis (median part of the lobus simplex and C_2 of

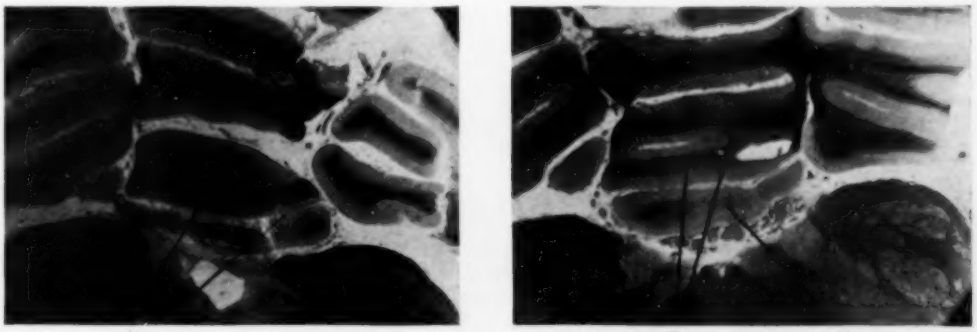

FIG. 2a. and 2b. Cat. 8.
D = Demyelination. G = Granular layer, necrotic.

Bolk, or lobus medius medianus of Sven Ingvar) was much less pronounced and lasted only a few hours after operation.

Experiment 2. (*Cat 38*). *Cauterization of the lobus medius medianus* (*June 26, 1941*) *followed by* ↑ *nystagmus with* ↶ *component in normal position* (frequency 2, 2, 3 in successive 10 sec. intervals) *and nystagmus toward the lower lid with* ↶ *component or purely* ↶ *nystagmus in supine position* (frequency 15, 9, 9, in successive 10 sec. intervals).

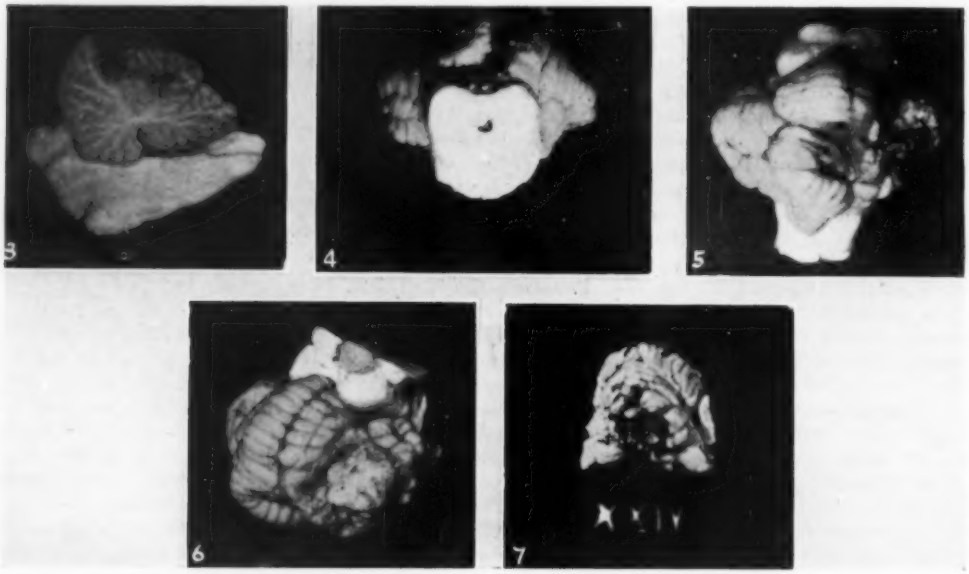

FIG. 3. Cat. 38.
FIG. 4. Cat. 28.
FIG. 5. Cat 36.
FIG. 6. Cat 27.
FIG. 7. Cat 24. Lateral view of the right cerebellar hemisphere.

Postoperative notes. First day (June 26). Four hours after the operation there was no nystagmus noticeable in any position.

The anatomic examination revealed a lesion reaching from the anterior part of the tuber to the most caudal lobules of the lobus anterior (Fig. 3).

As for the lobus anterior, small lesions affecting only parts of the lobus centralis and culmen were ineffective. Definite positional nystagmus appeared after extensive lesions of these lobules, but outlasted the operation for a few hours only.

Experiment 3 (*Cat 38*). *Cauterization of the lobus centralis and culmen except the most lateral parts on the left side.* Feb. 24, 1941, 11–12 a.m. *Transitory positional nystagmus.*

Postoperative notes. First day (Feb. 24). Half an hour after operation there was no nystagmus in *normal position*. In *right side position* there was nystagmus chiefly to the left canthus, while some jerks were directed toward the upper lid (frequency in successive 10 sec. intervals 18, 18, 14). In *left side position* there was nystagmus of large amplitude to the right canthus (frequency in successive 10 sec. intervals 19, 12, 8). In *supine position* there was nystagmus of smaller amplitude toward the lower lid (frequency in successive 10 sec. intervals 18, 16, 10). Three hours after operation the eyeballs deviated only occasionally

to the right, in normal position of the animal. There were no ocular movements in right or left side position, and in supine position only occasional eye jerks of very small amplitude toward the lower lid.

Anatomic examination: Extensive destruction of the cortex of the lobus centralis and culmen sparing only the most lateral parts on the left side. (Fig. 4)

Still less pronounced were the effects of lesions of the ansiform lobe. In some cases a definite nystagmus failed to appear at all despite extensive lesions of Crus I and II of this lobe (*e.g.*, Cat 36, Fig. 5); in others a weak positional nystagmus appeared immediately following the operation, *e.g.*, in cat 27 cauterization of the right ansiform lobe (Fig. 6); 10 minutes after operation: no definite nystagmus in normal position; ↷ Ny. in left side position; horizontal undulation in right side position; ↶ Ny. in supine position. No nystagmus 3 hours after operation.

As was pointed out by ten Cate (13) it is rather difficult to place strictly isolated lesions of the paraflocculus.* We were, however, able to produce lesions of the paraflocculus with only rather small associated lesions of the Crus I or Crus II of the ansiform lobe. In these cases the appearance of positional nystagmus was somewhat more pronounced than after extensive lesion of the ansiform lobe alone.

Experiment 4. (Cat 24). Cauterization of the dorsal and ventral part of the right paraflocculus. Jan. 17, 1941. 11:30 a.m.–12:20 p.m. *After operation the eyes showed occasional ↑ jerks in normal position, ↷ nystagmus in left lateral position, no definite nystagmus in supine position and right side position.*

Postoperative notes. First day (Jan. 17). Three hours after operation the animal showed hypermetric movements of the right hind limb when walking. There was no nystagmus in *normal position.* In *left lateral position* occasional ↷ jerks of small amplitude appeared. In *right lateral position* there were slow jerks to the lower lid and somewhat to the right (frequency in successive 10 sec. intervals 4, 4, 2, 3). In *supine position* nystagmic jerks toward the lower lid appeared (frequency in successive 10 sec. intervals 3, 0, 2, 2).

Second day (Jan. 18). Nystagmus could still be demonstrated in right lateral and in supine position, but not in normal and left lateral position. In *right lateral position* it was directed toward the lower lid, sometimes combined with a ↷ component, and had a frequency of 7, 6, 6, jerks in successive 10 second intervals. In *supine position* it was purely ↷ of small amplitude and had a frequency of 4, 5, 6 jerks in successive 10 second intervals.

Anatomic examination: There was a lesion of the dorsal and ventral part of the right paraflocculus encroaching upon the right crus secundum (Fig. 7).

It should be emphasized that positional nystagmus appeared in lesions of the cerebellar vermis, particularly the posterior part, although the medulla oblongata and pons were intact or showed on histological examination of serial sections only small foci of necrosis in the most caudal levels outside the vestibular nuclei and their fiber connections with the eye muscle nuclei (see protocol cat 8).

Since the cerebellum may influence the vestibular nuclei, chiefly by tecto-bulbar systems, special attention was paid to the question whether the lesions must encroach upon the nuclei tecti in order that nystagmus ap-

* In view of the smallness of the flocculus in cats and the concealed position of this structure underneath the paraflocculus on the side of the cerebellar peduncles, cats seem unsuitable for placing isolated lesions of the flocculus, and the influence of this structure upon positional nystagmus will have to be studied in other animals.

pears. In a number of cases positional nystagmus was observed, although the histologic examination failed to reveal definite alterations in the nuclei tecti. This does not exclude, however, the possibility that these nuclei may have been at least temporarily impaired by disturbance of their blood supply. If the positional nystagmus following lesions of the vermis should be caused by a temporary functional impairment of the nuclei tecti, one should expect that direct lesion of these nuclei should produce more pronounced and lasting effects. It seemed, therefore, of interest to compare the effect of lesions of the vermis with that of lesions of the nuclei tecti. These experiments showed that definite positional nystagmus may be produced by injury to these nuclei, but that it is neither more pronounced nor of longer duration than after lesions limited to the vermis (see following protocol).

Experiment 5 (*Cat 39*). *Destruction of the nuclei tecti produced positional nystagmus.*

Preoperative notes. 6-30-41. There was no positional nystagmus. Rotation on Bárány chair in normal position (extreme values of several tests):

10 × ↷: Nystagmus to left, 10–21 jerks in 11–12 seconds.
10 × ↶: Nystagmus to right, 15 jerks in 10–11 seconds.

Operation. July 2, 1941. 10:15–11:15 a.m. The nuclei tecti were electrolytically destroyed by a needle which was introduced in horizontal direction in the midline and at 1.5 mm. on either side of the midline 6 and 7 mm. above the calamus scriptorius into a depth of 10 mm.

Postoperative notes. First day (July 2). The animal showed opisthotonus, the forelimbs were in extended position. In *normal position* there was a nystagmus ↓ of small amplitude, 5 jerks in 30 sec. In *supine position* there was a nystagmus of medium amplitude toward the lower lid, sometimes with ↶ component. Frequency in successive 10 second intervals: 10, 10, 11, 15, 16, 16, 16, 23, 13; then the eyeballs rotated toward the lower lid and the nystagmus became undiscernible. In *right side* position there was nystagmus of medium amplitude toward the lower lid. Frequency in successive 10 sec. intervals: 10, 11, 5, 6. In *left side* position there was nystagmus toward the lower lid of small amplitude. Frequency in successive 10 sec. intervals: 8, 6, 6, 5, 5.

Second day. (July 3). Opisthotonus and extended position of the forelimbs were still present. While in *normal position* no nystagmus appeared, the animal showed in *supine position* nystagmus toward the lower lid. Frequency in successive 10 sec. intervals: 11, 10, 9, 9. 14, 15. In *left side position* nystagmus ↷ and toward the lower lid, 5 jerks in 30 sec. In *right side position* there was nystagmus toward the lower lid. Frequency in successive 10 second intervals: 5, 6, 8, 8. Rotation on Bárány chair in normal position. (Extreme values of several tests):

10 ↷: Nystagmus to left, 29–32 jerks in 15–19 seconds.
10 ↶: Nystagmus to right, 25–30 jerks in 13–15 seconds.

Fourth day. (July 5). Opisthotonus was still present, but no nystagmus could be detected in *normal and left side position.* In *supine position* there was nystagmus toward the lower lid of medium, then of small amplitude. Frequency in successive 10 sec. intervals: 0, 0, ?, 3, 3, 5, 4, 4, 2, 3. In *right side position* there was nystagmus of small amplitude to the lower lid, sometimes . Frequency in successive 10 sec. intervals: 1, 3, 2, 3, 2.

Eighth day (July 9). While no definite opisthotonus could be found, the forelimbs were still held in somewhat extended position when the animal walked. There was no nystagmus in *normal* and *right side* position, but occasional jerks could be noticed in *supine position,* (2, 1, 2 in successive 30 sec. intervals, toward upper lid and ↷) and *in left side position* (1, 0, 0, jerks in 30 sec. intervals in ↶ direction). Rotation on Bárány chair in normal position (Extreme values of several tests):

10 ↷: Nystagmus to left, 18–24 jerks in 10–12 seconds.
10 ↶: Nystagmus to right, 16–18 jerks in 8–12 seconds.

Tenth day (July 11). Thère were only occasional eye movements (0–3 per 30 sec.) in

all positions of the animal. Rotation on Bárány chair in normal position. (Extreme values of several tests).

10↷: Nystagmus to left, 15–17 jerks in 10 seconds.
10↶: Nystagmus to right, 11–15 jerks in 8–9 seconds.

Eleventh day (July 12). There was no nystagmus in any position. The animal was sacrificed.

Histologic examination: Small puncture canals pass through pyramis and uvula; they end in electrolytic lesions destroying the nuclei tecti (Fig. 8) and the right nucleus globosus and encroach upon the left nucleus globosus.

Control experiments showed that normal or anesthetized cats do not show positional nystagmus. Immediately after awakening from ether anesthesia, there may exist a weak spontaneous nystagmus or undulation in

FIG. 8. Cat 39.

normal position. Change of position does not increase this nystagmus, or at most occasionally a few jerks appear immediately after the animal has been turned into an abnormal position (*e.g.*, lateral position).

Characteristics of the positional nystagmus. The positional nystagmus is usually most marked in supine position of the animal, occasionally it is more pronounced in side position, (*e.g.*, sometimes after paraflocculer lesions or asymmetric lesion of the vermis or of the nuclei tecti). Its appearance is often preceded by a latent period of a few seconds after the animal has been turned from the normal into the abnormal position. Sometimes, however, jerks appear immediately after the new position has been obtained.

The change from the normal into the abnormal position may not only increase frequency and amplitude of the nystagmus but also influence its

direction. In *normal* position one usually observes in lesions of the vermis, if reactions of the eyeballs appear at all, a downward deviation of the eyeballs and/or a preponderantly vertical nystagmus of low frequency with the fast component to the upper or lower lid as previously described. After the animal has been brought into the *supine position*, there usually appears a nystagmus toward the lower lid of higher frequency and sometimes also larger amplitude than in the normal position. Thus nystagmus toward the upper lid in normal position may be reversed in its relation to the orbit. Combination with a deviation of the eyeballs chiefly towards the lower lid and/or with a rotary component of the nystagmus on one or both eyes could be observed in normal as well as in supine position. In *side position* the nystagmus usually beats parallel to the palpebral fissure or somewhat obliquely, with the fast component to the upper ear. Or rotary nystagmus is observed, *e.g.*, ↷ in left side position and ↶ when the animal lies on the right side.

If one compares successive 10-second intervals, frequency and often also amplitude of the nystagmus diminish rather rapidly after the animal has been placed into the abnormal position. Repeated testing (turning from the normal into abnormal positions) without pause sometimes also produces exhaustion of the reaction so that it may be more pronounced on the first examination than on the subsequent ones.

It seems particularly noteworthy that in a number of cases nystagmus appeared only in abnormal position of the animal (head), while it was absent in the normal position. This could particularly be observed when small lesions had been placed (see Cat 8).

In all experiments the positional nystagmus was only transient and disappeared within about a week following operation. As was already mentioned, this was found not only following lesions limited to the cerebellar cortex and adjacent white matter, but also in lesions of the nuclei tecti (see protocol Cat 39).

Influence of various receptors. As pointed out in the description of the method of examination, neck reflexes were avoided by keeping the head and body of the animal in fixed relative position on the animal board. In a number of experiments retinal impulses were eliminated by covering the anesthetized cornea with a small rubber membrane that was blackened with India ink. This had no effect upon the positional nystagmus. Finally bilateral labyrinthectomy was performed in animals which showed typical positional nystagmus following lesions of the median part of the posterior lobe, as shown by the following protocol.

Experiment 6 (Cat 18). Destruction of uvula and lesion of ventral part of pyramis cerebelli followed by positional nystagmus. After additional bilateral labyrinthectomy only weak spontaneous nystagmus, practically not influenced by changes in position.

First operation (Dec. 9, 1940. 10:40–11:20 a.m.). The uvula cerebelli was mechanically extirpated under ether anesthesia.

Postoperative notes: First day (Dec. 9). At 3:00 p.m. there was tendency to opisthotonus and increased extensor tone of the limbs. In *normal position* a nystagmus of small amplitude

↑ and ↶ was observed (7–9 jerks in 30 sec.). In *supine position* there was nystagmus toward the lower lid and slightly ↶ (31–37 jerks in 30 sec.). In *right side position* there was nystagmus ↶ of very small amplitude (35–36 jerks in 30 sec.). In *left side position* there was nystagmus of small amplitude toward the lower lid (25–27 jerks in 30 sec.).

Second operation 3:45–4:15 p.m. Bilateral labyrinthectomy.

Postoperative notes: First day (Dec. 9) 4:50 p.m. In *normal position* the eyes showed slow vertical ↓ movements, some with ↶ component, occasionally with ← component to the right. In *supine position* there were eye jerks toward the lower lid, some with component to right, 4–6 in 30 sec. In *right side position* there were 5 ↶ jerks to the right in 30 sec. In *left side* position there were 1–4 ↓ and ↶ jerks in 30 sec. Rotation on a Bárány chair: 10 × to the right, then 10 × to the left did not influence the spontaneous ↓ jerks.

Second day (Dec. 10). In *normal position* the eyes showed ↓ and ↶ jerks, 3–8 in 30 sec. In *supine position* there were eye jerks toward the lower lid and ↶; 4–7 jerks in 30 sec. In *left side position* there were slight ↶ eye jerks, 1–4 in 30 sec. In *right side position* there were also ↶ jerks, 1–5 in 30 sec. Rotation on Bárány chair, 10 × to the right, then to left did not influence the spontaneous ↓ and ↶ jerks.

Animal sacrificed.

Anatomic examination: Destruction of the uvula and lesion of the ventral part of the pyramis cerebelli. The inner ear is replaced on both sides by a large cavity that communicates with the internal auditory meatus.

The absence of excitable receptors in the labyrinth was shown by the ineffectiveness of rotation on a Bárány chair. Despite the elimination of the labyrinths a weak spontaneous nystagmus (a few jerks per min.) could still be observed; this was apparently of central origin as will be pointed out later. Changes of position did not definitely influence this nystagmus beyond the variations that it showed spontaneously in normal position.

Discussion

Little is known regarding the influence of the cerebellum upon vestibular reflex arcs in oblongata and pons. Lesion of the vermis-nuclei tecti system may produce, at least in some species, transient overexcitability of these reflex arcs (increase of duration and intensity of experimental nystagmus observed by Bauer and Leidler (14) in rabbits; increase of tonic labyrinthine reflexes upon limb and trunk muscles described by Pollock and Davis (15) in decerebrate cats; negative results reported by Dow (16) in monkeys and chimpanzees after subtotal ablation of uvula and nodulus).

In this group of symptoms of overexcitability of vestibular reflex arcs following cerebellar lesions one may perhaps also include the observations reported in this paper. Such an interpretation is supported by experiments in which we tested not only positional nystagmus but also experimental nystagmus produced by rotation on a Bárány chair before and after the cerebellar operation.

In Fig. 9 the results of such examinations of the excitability of the labyrinth are charted for Cat 39 (lesion of the nuclei tecti, see experiment 5). As an indication of the intensity of the positional nystagmus the number of jerks per 30 sec. in supine and in right side position was chosen. One may notice that the curves of the positional nystagmus and of the postrotatory nystagmus reach their maximum on the same day; although their descend-

ing course is not strictly parallel, they reach the normal level at the same time.

If these experiments are surveyed from the point of view of localization, the phenomenon of positional nystagmus is most marked after lesions of the lobus posterior medianus but may also appear, although less marked and of still shorter duration, after lesions of other parts of the cerebellum, particularly palaeo-cerebellar areas.

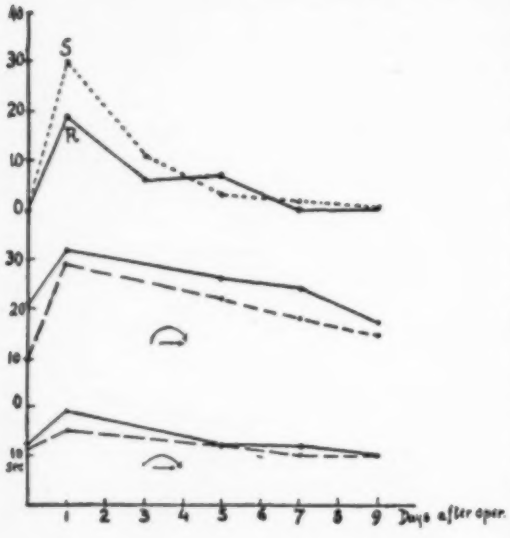

FIG. 9. Experimental postrotatory and positional nystagmus before and after lesion of the nuclei tecti. (Cat 39).

Nystagmus ⇄ to the left after 10 rotations to the right;
Minimal number of jerks..... – – –
Maximal number of jerks..... ———
Minimal duration..... – – –
Maximal duration..... ———
Position nystagmus in supine position (S ·····) and in right side position (R ———).
Number of jerks in 30 sec.

The question can only partly be answered why the appearance of positional nystagmus depends on the lesion of certain cerebellar areas. One should expect that such an effect is observed after extirpation of such parts of the cerebellum as send efferent fibers to the vestibular nuclei. Thus one can easily understand that lesions of areas which send no or only scanty impulses to these nuclei such as the ansiform lobe are followed by a positional nystagmus of a few hours duration only. The same may apply to the lobus medius medianus whose connections with the nuclei tecti seem less developed than those of the other parts of the vermis (see fig. 93 in Fulton, 17). The definite effect of lesions of the lobus posterior medianus may be explained by the fact that this lobe sends efferent impulses to the vestibular nuclei not only by way of the tectobulbar system but also by way of direct fibers to the vestibular nuclei (from the uvula and nodulus, 18). As for the paraflocculus, Clarke and Horsley (19) described efferent connections with the nuclei tecti, while Dow traced degenerating fibers to the dentate nucleus and nucleus interpositus.

It may seem surprising that extensive lesions of the lobus centralis and culmen of the anterior lobe* produce a positional nystagmus of much shorter duration than lesions of the posterior lobe, although these parts of the anterior lobe send also efferent fibers to the nuclei tecti. Various factors may contribute to this difference. First the possibility should be borne in mind

* The anterior lobe will be discussed here with exception of the lingula, to which a special study shall be devoted.

that lesions of the posterior lobe may more easily affect the nuclei tecti by interfering with their vascular supply than do lesions of the anterior lobe. In view of the fact that direct injuries to the nuclei tecti do not produce a more pronounced and longer lasting positional nystagmus than lesions of the vermis, this factor should not be overestimated.

Second, the lesion of direct efferent fibers from the cortex of the vermis to the vestibular nuclei may play a more important role than the lesion of the cortico-tectal connections. Such direct cortico-vestibular fibers were chiefly traced from the lobus posterior medianus as previously mentioned. According to Saito (20), however, all parts of the vermis, and also the lateral lobes of the cerebellum send direct efferent fibers to the region of the Deiter's nucleus by way of the fibrae perforantes.

Third, the possibility should be considered that the cerebellofugal systems to the rhombencephalon may be functionally differentiated in that certain parts may act chiefly upon the postural reflex arcs and others chiefly upon the vestibulo-ocular reflex arcs. This may apply to the direct cerebello-bulbar fibers (*e.g.*, fibrae perforantes) as well as to the fastigio-bulbar systems. There are indications that the part of these systems originating from the lobus centralis and culmen influences the postural centers of the limb and neck muscles in oblongata and pons. Sherrington (21) and others showed that the extensor rigidity in the limbs of decerebrate cats may be inhibited by stimulation of the anterior lobe only; this reaction can be maintained by cerebello-bulbar systems alone, since it persists after transverse section behind the midbrain (22, 23). In agreement with the stimulation experiments, lesions of lobus centralis and culmen produced only a release of the neck extensors and of the antigravity muscles of the limbs (24). Thus it seems understandable that lesion of these areas have only a slight effect in producing positional nystagmus.

It may be of interest to compare our results with the present trend of cerebellar physiology. A sharp localization of single muscle groups within the various lobules of the cerebellar cortex, as suggested by Bolk and by Rynberk, is not supported by recent stimulation experiments of Hare, Magoun and Ranson (25). This does not exclude, however, a functional localization as proposed by Fulton (17). Our observations seem to agree with such a scheme of localization with the restriction that a functional group such as the vestibular nuclei is influenced chiefly but not exclusively by a certain area of the cerebellar cortex.

The question may be raised whether the influence of changes in the position of the head upon the spontaneous nystagmus, particularly upon its direction, is due to reflex changes in the state of tonic contraction of certain eye muscles produced by tonic labyrinthine reflexes upon these muscles. The nystagmus could of course show a change of direction if it were superimposed upon tonic reflex reactions of the eye muscles. Tonic labyrinthine reflexes upon the eyes play, however, only a minor role in animals with frontal position of the eyeballs in which the visual fields overlap, and the

position of the eyeballs chiefly depends on retinal reflexes (26). In our cats with cerebellar lesions a deviation of the eyeballs to the lower lids was sometimes more pronounced in supine than in normal position; positional nystagmus, however, appeared with as well as without tonic deviation of the eyeballs.

Thus changes in the state of contraction of eye muscles due to labyrinthine reflexes upon these muscles played at the most an accessory part in the mechanism of the positional nystagmus after cerebellar lesions. Static impulses from the labyrinth could, however, act in the center upon the kinetic part of the vestibulo-ocular reflex arc. Lorente de Nó (27) observed on otherwise normal rabbits that a nystagmus produced by rotation or by caloric stimuli may be modified as to intensity, and sometimes also as to direction, by changes in the position of the head although the same stimuli were produced in the semicircular canals. There occurred in these cases apparently an interaction of impulses from the static receptors with those from the semicircular canals. In our experiments we have to do with a nystagmus that persisted, at least to a certain extent, after bilateral labyrinthectomy and was apparently due to an abnormal discharge of the vestibular nuclei that were in a state of increased excitability after the cerebellar operation. One may perhaps assume that in this state of overexcitability there occurs not only an increase of discharges from the vestibular nuclei to the eye muscles resulting in "spontaneous nystagmus," but also a spread of impulses originating in the static receptors of the labyrinth to those parts of the vestibular nuclei which transmit kinetic reactions to the eye muscles. Due to such a spread, impulses from the static receptors may modify intensity and also direction of a "spontaneous nystagmus" or may even cause its appearance in certain positions of the head.

In agreement with such an explanation our experiments showed that changes in the position of the head were no longer able to influence the spontaneous nystagmus after the labyrinths had been eliminated. The fact that a weak "spontaneous nystagmus" may persist for some time after bilateral labyrinthectomy may seem astonishing. The appearance of Bechterew's compensatory nystagmus, when some days after extirpation of the first labyrinth, the second labyrinth is also eliminated, and the observation of nystagmus following punctures of the vestibular nuclei in labyrinthectomized dogs (28) show, however, that the vestibular nuclei are still able to discharge after elimination of the peripheral receptors.

The experience was mentioned that in some of our animals nystagmus was present in abnormal positions only. This seems to suggest that the examination of patients with suspected cerebellar lesions should routinely include an examination of positional nystagmus. Such an examination may yield positive results in certain positions despite the absence of spontaneous nystagmus in normal position.

Summary

After lesion of parts of the cerebellum of cats, particularly of the lobus posterior medianus, nystagmus was observed in abnormal positions of the head, particularly in supine position with the vertex downward. A weak nystagmus in normal position of the head was increased, sometimes also its direction was changed, in abnormal positions; or nystagmus appeared in abnormal positions only. This phenomenon is transient. Electrolytic lesions of nuclei tecti had a similar effect. In analyzing the receptor mechanism, neck reflexes and retinal reflexes could be excluded. Bilateral labyrinthectomy abolished this effect of position, while a weak spontaneous nystagmus could persist. It is assumed that the appearance of positional nystagmus following cerebellar lesions is a phenomenon of release of parts of the vestibulo-ocular reflex arcs, since it is associated with increase of the experimental postrotatory nystagmus. The experiments suggest that the existence of positional nystagmus should routinely be tested in suspected cerebellar lesions.

REFERENCES

1. Bárány, R. Nervöse Stoerungen des Cochlear und Vestibular-apparates. *Lewandowsky's Handb. Neurol.*, 1910, *1:* 919–958.
2. Bauer, J., and Leidler, R. Einfluss der Ausschaltung verschiedener Hirnabschnitte auf die vestibul. Augenreflexe. *Arb. Neurol. Inst. Wien.*, 1912, *19:* 155–226.
3. Dusser de Barenne, J. G. Funktionen des Kleinhirns, *Handb. Neurol. Ohres.*, 1923, *1:* 589–672.
4. Goldstein, K. Kleinhirn. *Handb. norm. pathol. Physiol.* 1927, *10:* 222–317.
5. Marburg, O. Tumoren im Bereich des Cochlear und Vestibularsystems. *Handb. Neurol. Ohres.*, 1926, *3:* 1–166.
6. Holmes, G. The symptoms of acute cerebellar injuries. *Brain.* 1917. *40:* 461–535.
7. Oppenheim, H. *Lehrbuch der Nervenkrankheiten.* 7th ed. Berlin, S. Karger, 1923. 2 vol.
8. Weisenburg, Th. Cerebellar localization. *Brain*, 1927, *50:* 357–377.
9. Sachs, E. *Diagnosis and treatment of brain tumors.* C. V. Mosby, Co., St. Louis, 1931. 381 pp.
10. Nylén, C. O. Clinical study on positional nystagmus in cases of brain tumor. *Acta oto-laryngol.*, 1931, *15* (Suppl.): 113 pp.
11. Ruttin, E. Neuritis cochlearis und vestibularis. *Mschr. Ohrenheilk.*, 1930, *64:* 5 3.
12. Spiegel, E. and Scala, N. Vertical nystagmus in cerebellar lesions. *Arch. Ophthalm., N. Y.*, 1941, *26:* 661–669.
13. ten Cate, J. Le paraflocculus. *Arch. néerl. Physiol.*, 1926, *10:* 24–53.
14. Bauer, J., and Leidler, R. Einfluss der Ausschaltung verschiedener Hirnabschnitte auf die vestibulären Augenreflexe. *Arb. Neurol. Inst. Wien.*, 1912, *19:* 155–226.
15. Pollock, L. J., and Davis, L. Influence of the cerebellum upon the reflex activities of the decerebrate animal. *Brain*, 1927, *50:* 277–312.
16. Dow, R. S. Effect of lesions in vestibular part of cerebellum in primates. *Arch. Neurol. Psychiat., Chicago*, 1938, *40:* 500–530.
17. Fulton, J. F. *Physiology of the nervous system.* Oxford Univ. Press, New York, 1938. 675 pp.
18. Dow, R. S. Fiber connections of the posterior part of the cerebellum. *J. comp. Neurol.*, 1936. *63:* 527–548.
19. Clarke, R. H., and Horsley, V. Intrinsic fibers of the cerebellum. *Brain*, 1905, *28:* 13–29.
20. Saito, M. Experimentelle Untersuchungen ueber die inneren Verbind. der Kleinhirnrinde. *Arb. Neurol. Inst. Wien*, 1922, *23:* 74–106.
21. Sherrington, C. S. Decerebrate rigidity and reflex coordination of movements. *J. Physiol.*, 1898, *22:* 319–332; Parts of the brain below cerebral cortex, in: *Schäfer, Textbook of physiology*, 1900, *2:* 884–919.

22. BERNIS, W. B., and SPIEGEL, E. A. Zentren der statischen Innervation. *Arb. Neurol. Inst. Wien.*, 1925, *27:* 197–224.
23. HARE, W. K., MAGOUN, H. W., and RANSON, S. W. Electrical stimulation of the interior of the cerebellum in the decerebrate cat. *Amer. J. Physiol.*, 1936, *117:* 261–266.
24. CONNOR, G. J. Functional localization within the anterior cerebellum. *Proc. Soc. exp. Biol., N. Y.*, 1941, *47:* 205–207.
25. HARE, W. K., MAGOUN, H. W., and RANSON, S. W. Localization within the cerebellum. *J. comp. Neurol.*, 1937, *67:* 145–182.
26. MAGNUS, R., and DE KLEIJN, A. Experimentelle Physiologie des Vestibularapparates. *Handb. Neurol. Ohres.*, 1923, *1:* 465–552.
27. LORENTE DE NÓ, R. Ausgewählte Kap. aus der vergleichenden Physiologie des Labyrinthes. *Ergebn. Physiol.*, 1931, *32:* 73–242.
28. SPIEGEL, E. and SATO, G. Erregungszustand der vestibulären Zentren nach doppelseitiger Labyrinthausschaltung. *Pflüg. Arch. ges. Physiol.*, 1926, *215:* 106–119.

EFFECT OF ALKALOSIS AND ACIDOSIS ON CORTICAL ELECTRICAL ACTIVITY AND BLOOD FLOW*

ALBERT J. LUBIN† AND J. C. PRICE

Neurological Institute of New York and the Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

(Received for publication March 19, 1942)

A DECREASE in CO_2 tension of the blood causes a decrease in frequency and an increase in amplitude of cortical potentials while an increase in CO_2 tension has the opposite effect (2, 6, 9). Whether these changes are directly a function of the CO_2 or are related to variations in the hydrogen ion concentration of the blood has not been clearly demonstrated. The common use of the terms "alkalosis" and "acidosis" are tacit acknowledgement that they are related to the latter. Davis and Wallace (3) have shown that the acidosis or alkalosis produced by oral ingestion of sodium bicarbonate and ammonium chloride respectively cause no significant change in the effect of hyperventilation on the electroencephalogram of man. They suggest that hyperventilation causes the appearance of delta waves by inducing cerebral vasoconstriction, thereby diminishing the supply of oxygen and glucose to the cortex.

This study is an attempt to determine (i) the effect of alkalosis and acidosis produced by the intravenous injection of chemicals on the cortical electrical activity of the anesthetized cat; (ii) the relationship of the respiratory changes in alkalosis and acidosis to the electrocorticographic changes; and (iii) the role of cerebral blood flow in the production of changes in cortical potentials due to alkalosis and acidosis. This was attempted by means of the simultaneous recording of cortical potentials and the measurement of pial vessel size through a skull window.

METHODS

The cats were firmly fixed on a heated animal holder after being anesthetized with pentobarbital sodium (approximately 0.03 g. per kg. of body weight intraperitoneally). A Forbes' lucite window was screwed into a threaded trephine opening after exposing an area of cortex in the anterior portion of the parietal lobe. A silver-tipped screw was inserted into one of the openings in the window, thus serving both as a monopolar electrode and a seal for preventing the escape of cerebrospinal fluid. The indifferent electrode was placed on the ear. Electrical activity was recorded with electro-dynamic ink writers driven by a resistance-capacity coupled amplifier (time constant = 0.25 sec.). The input stage of the amplifier was of the differential type thus facilitating recording with a minimum of shielding.‡ Photographs of the pial vessels were taken through the skull window at frequent intervals with the use of a Leitz Ultropak microscope and a reflex camera, using 35 mm. panchromatic film. The negatives were subsequently enlarged and measurements made of arteries ranging in size from 50 to 250 micra in diameter.

* This study was aided by a grant from Parke, Davis and Co.

† Fellow of the Rockefeller Foundation.

‡ Mr. C. W. P. Walter designed and constructed the recording apparatus.

22. BERNIS, W. B., and SPIEGEL, E. A. Zentren der statischen Innervation. *Arb. Neurol. Inst. Wien.*, 1925, *27:* 197–224.
23. HARE, W. K., MAGOUN, H. W., and RANSON, S. W. Electrical stimulation of the interior of the cerebellum in the decerebrate cat. *Amer. J. Physiol.*, 1936, *117:* 261–266.
24. CONNOR, G. J. Functional localization within the anterior cerebellum. *Proc. Soc. exp. Biol., N. Y.*, 1941, *47:* 205–207.
25. HARE, W. K., MAGOUN, H. W., and RANSON, S. W. Localization within the cerebellum. *J. comp. Neurol.*, 1937, *67:* 145–182.
26. MAGNUS, R., and DE KLEIJN, A. Experimentelle Physiologie des Vestibularapparates. *Handb. Neurol. Ohres.*, 1923, *1:* 465–552.
27. LORENTE DE NÓ, R. Ausgewählte Kap. aus der vergleichenden Physiologie des Labyrinthes. *Ergebn. Physiol.*, 1931, *32:* 73–242.
28. SPIEGEL, E. and SATO, G. Erregungszustand der vestibulären Zentren nach doppelseitiger Labyrinthausschaltung. *Pflüg. Arch. ges. Physiol.*, 1926, *215:* 106–119.

EFFECT OF ALKALOSIS AND ACIDOSIS ON CORTICAL ELECTRICAL ACTIVITY AND BLOOD FLOW*

ALBERT J. LUBIN† AND J. C. PRICE

Neurological Institute of New York and the Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

(Received for publication March 19, 1942)

A DECREASE in CO_2 tension of the blood causes a decrease in frequency and an increase in amplitude of cortical potentials while an increase in CO_2 tension has the opposite effect (2, 6, 9). Whether these changes are directly a function of the CO_2 or are related to variations in the hydrogen ion concentration of the blood has not been clearly demonstrated. The common use of the terms "alkalosis" and "acidosis" are tacit acknowledgement that they are related to the latter. Davis and Wallace (3) have shown that the acidosis or alkalosis produced by oral ingestion of sodium bicarbonate and ammonium chloride respectively cause no significant change in the effect of hyperventilation on the electroencephalogram of man. They suggest that hyperventilation causes the appearance of delta waves by inducing cerebral vasoconstriction, thereby diminishing the supply of oxygen and glucose to the cortex.

This study is an attempt to determine (i) the effect of alkalosis and acidosis produced by the intravenous injection of chemicals on the cortical electrical activity of the anesthetized cat; (ii) the relationship of the respiratory changes in alkalosis and acidosis to the electrocorticographic changes; and (iii) the role of cerebral blood flow in the production of changes in cortical potentials due to alkalosis and acidosis. This was attempted by means of the simultaneous recording of cortical potentials and the measurement of pial vessel size through a skull window.

METHODS

The cats were firmly fixed on a heated animal holder after being anesthetized with pentobarbital sodium (approximately 0.03 g. per kg. of body weight intraperitoneally). A Forbes' lucite window was screwed into a threaded trephine opening after exposing an area of cortex in the anterior portion of the parietal lobe. A silver-tipped screw was inserted into one of the openings in the window, thus serving both as a monopolar electrode and a seal for preventing the escape of cerebrospinal fluid. The indifferent electrode was placed on the ear. Electrical activity was recorded with electro-dynamic ink writers driven by a resistance-capacity coupled amplifier (time constant = 0.25 sec.). The input stage of the amplifier was of the differential type thus facilitating recording with a minimum of shielding.‡ Photographs of the pial vessels were taken through the skull window at frequent intervals with the use of a Leitz Ultropak microscope and a reflex camera, using 35 mm. panchromatic film. The negatives were subsequently enlarged and measurements made of arteries ranging in size from 50 to 250 micra in diameter.

* This study was aided by a grant from Parke, Davis and Co.

† Fellow of the Rockefeller Foundation.

‡ Mr. C. W. P. Walter designed and constructed the recording apparatus.

After preliminary trials with other chemicals and various concentrations, a 0.1 *N* solution of HCl and a 2.0 *N* solution of Na_2CO_3 were found to be the most suitable means of producing characteristic changes in the respiratory rate with a minimum of toxic effects. This work is based on 21 injections of HCl and 25 injections of Na_2CO_3 in 12 animals. Because of the buffering capacity of the blood, the rate of injection was an important factor; however the change in respiratory rate has been found to be an index of pH change following injections of acids or alkalis (7). The average amount of HCl injected was approximately 0.0001 moles per kg. of body weight and that of Na_2CO_3 was approximately 0.001 moles per kg. of body weight.

The electrical activity recorded from the cortex of the cat under pentobarbital sodium anesthesia consists of a series of high voltage bursts at a frequency of about 7 to 10 per sec. with little activity between the bursts. This is similar to that seen in dial anesthesia and probably affords the most reliable base line for experimental studies (1).

Results

a. *Acid injection:* Injections of amounts of HCl necessary to produce a definite increase in the respiratory rate in no case caused any definite change in cortical potentials (Fig. 1A). In most cases, moreover, when larger, near-fatal amounts were injected the only change noted was a disappearance of all activity. Occasionally, however, with these large amounts of acid, low voltage, fast activity (11–16 per sec.) was produced and the slower (7–10 per sec.) activity seen in bursts in normal anesthetized animals was ob-

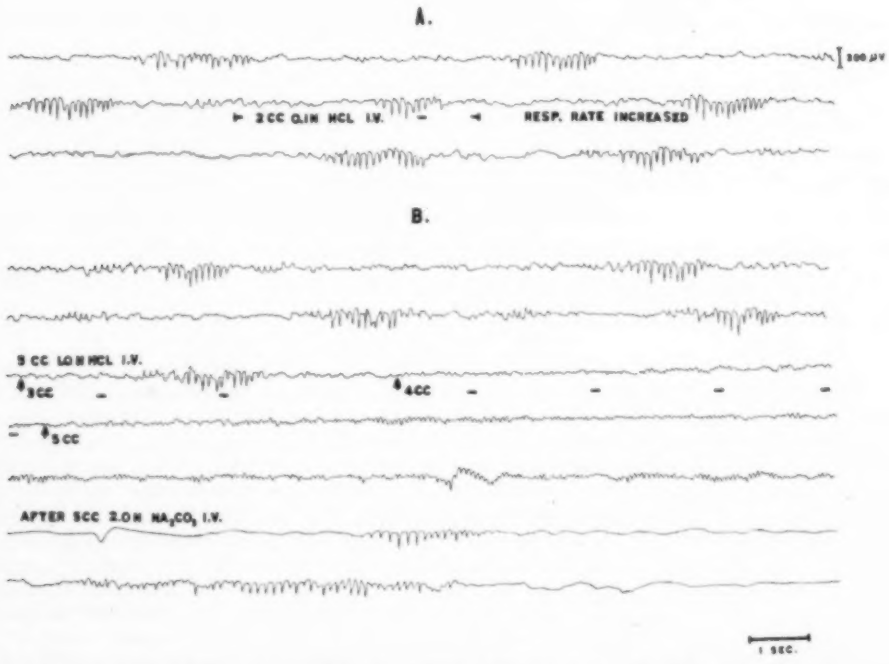

FIG. 1. A. No change is noted in the cortical potentials after injection of amounts of HCl that produce a definite increase in the respiratory rate. B. Injection of a near-fatal amount of HCl is followed by a train of rapid waves of low voltage. Subsequent injection of Na_2CO_3 results in the disappearance of these waves. After an interval in which no activity is recorded, the large amplitude waves seen in the normal record return.

literated (Fig. 1B). This rapid activity was noted, however, only when it was discernible to some extent in the record preceding the injections.

b. *Alkali injection:* Amounts of Na_2CO_3 necessary to produce a definite decrease in the respiratory rate and sometimes twitching and muscular hyperirritability ordinarily caused no definite change in cortical potentials (Fig. 2A and 3A). Occasionally, and even here usually with doses larger than required to produce respiratory changes, there was a transient obliteration

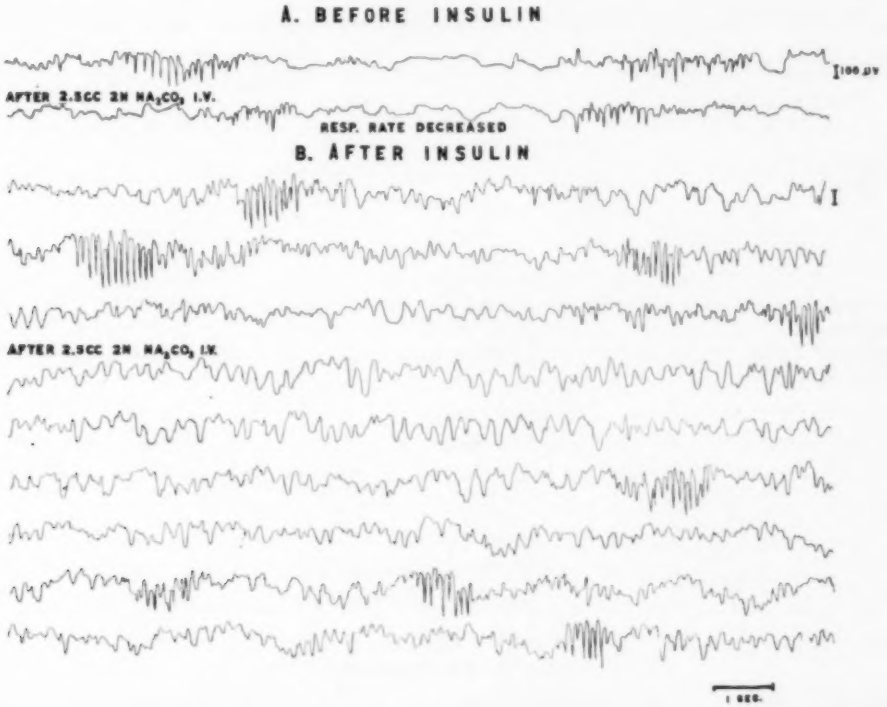

FIG. 2. A. Injection of amounts of Na_2CO_3 sufficient to produce a definite increase in the respiratory rate cause no remarkable change in cortical activity. B. About 90 minutes following the injection of 40 units of insulin many slow waves have made their appearance. After the injection of the same amount of Na_2CO_3 as in A there is a marked increase in the number of slow, high voltage waves (similar to those in Fig. 4A).

of all activity, possibly associated with anoxia. In one animal, however, in which much slow random activity was present (possibly due to trauma) injection of Na_2CO_3 in amounts that ordinarily produced no change, resulted in a series of smooth, high voltage 4–6 per sec. waves, similar to those sometimes seen after hyperventilation (Fig. 4). The injection was repeated four times with the same result.

This suggested the employment of insulin hypoglycemia as a means of changing the normal record and then injecting alkali. Preliminary injections at first produced no change in cortical activity (Fig. 2A and 3B). In the animal whose records are illustrated in Fig. 2 considerable slow activity,

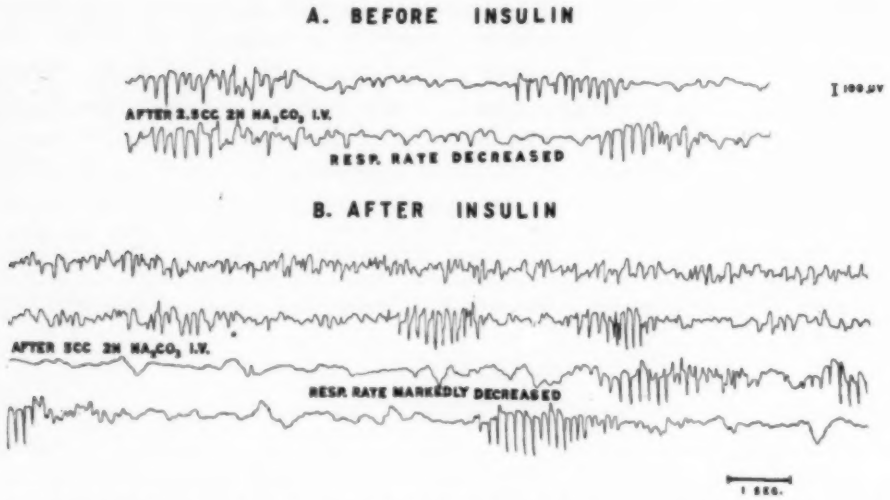

FIG. 3. A. Following the injection of Na_2CO_3 there is a decrease in respiratory rate with no change in cortical activity. B. One hour after the injection of 40 units of insulin Na_2CO_3 injection is followed by the disappearance of all activity except a few slow waves. The large amplitude bursts return before the smaller amplitude rapid activity which was recorded prior to the administration of Na_2CO_3.

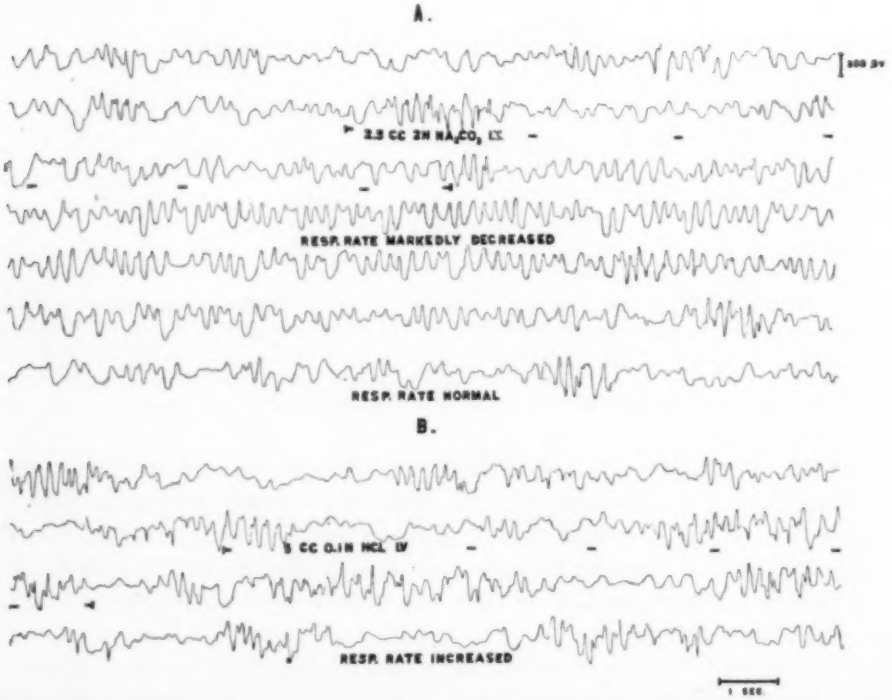

FIG. 4. A. Slow random activity and none of the ordinary bursts are present. Administration of Na_2CO_3 results in an increase in high voltage, four to six per second waves. B. Injection of HCl causes no remarkable change

some having a voltage of about 100–150μV, was present in addition to the usual bursts about 90 min. following the injection of 40 units of insulin. The injection of Na_2CO_3 in the same amounts as in the control period resulted in a series of rather regular, high voltage slow waves, usually four per second, (Fig. 2B), which were similar to those observed after the injection of Na_2CO_3 illustrated in Fig. 4A. Return of the electrical activity to a prealkalotic level occurred in about the same time in both instances. In another animal (Fig. 3) considerable fast activity of lower voltage was present about 60 minutes after the injection of insulin. Na_2CO_3 alkalosis caused all activity to disappear; the slower, high voltage bursts returned long before the faster waves,

Table 1. Effect of injections of hydrochloric acid and sodium carbonate solutions on the diameter of pial arteries

	Constriction					Per cent	Dilatation							Total		
	per cent						per cent									
	25	20	15	10	5	0	5	10	15	20	25	30	35	Constriction	No Change	Dilatation
HCl	0	0	0	0	0	5	4	4	2	3	0	0	2	0	5	15
Na_2CO_3	1	1	4	12	12	12	7	2	0	0	0	0	0	30	12	9

their frequency (8 per sec.) about the same but slightly larger in amplitude. Similar obliteration of fast activity by Na_2CO_3 is seen in Fig. 1B.

c. *Pial vessel size:* Twenty measurements of the diameter of pial arteries were made in 6 animals following 9 injections of hydrochloric acid solution. Fifteen of these measurements revealed vasodilatation ranging from 5 to 35 per cent. Five measurements revealed no change in vessel size; in four of these five the minimal amount of acid injected was 1 cc. of a 0.1*N* solution. In no case was vasoconstriction observed.

Fifty-one measurements of pial artery size were made in 9 animals following 22 injections of a solution of sodium carbonate. Vasoconstriction, from 5 to 25 per cent, was noted in thirty of these measurements; the constriction was 10 per cent or less in twenty-four. No change in vessel diameter was noted in twelve; vasodilatation of 5 to 10 per cent was noted in nine.

Vasodilatation following the injection of other acids and vasoconstriction following injection of other bases have frequently been observed (13). According to Poiseuille's Law the rate of flow is proportional to the fourth power of the diameter of a tube. If this law is applicable to cerebral blood flow (as it seems to be elsewhere in the body, 5) these relatively small changes in diameter represent marked changes in blood flow, as illustrated in the table at the top of the next page.

These changes in vessel diameter which probably represent significant changes in blood flow occurred without relationship to variations in the cortical potentials simultaneously recorded. In fact, the slow waves shown in Fig. 2B following injection of sodium carbonate were accompanied by a

Table 2. Change in blood flow according to Poiseuille's law.

Per cent change in diameter	Flow (100 = Initial flow)	
	vasodilatation	vasoconstriction
5	122	81
10	146	66
15	175	52
20	207	41
25	244	32
30	286	24
35	332	18

slight increase in diameter of the pial vessels. This does not tend to support the suggestion of Davis and Wallace that slow waves following hyperventilation are due to vasoconstriction and cerebral anemia.

Discussion

It seems clear, at least in the case of the agents used here, that there is no direct relationship between the respiratory rate and the frequency of cortical potentials in normal anesthetized animals. Marked respiratory changes may be present with no appreciable alteration in the electrocorticogram. When alterations in frequency do occur, however, they tend to occur in the same direction as the respiratory changes, as suggested by Gibbs and Gibbs (8); that is, a decrease with alkalosis and an increase with acidosis. The cortex seems to be more stable in the presence of changes of pH than does the respiratory center. The chemo-receptors in the carotid body (10) may contribute to this difference, there being no evidence at present for any such accessory mechanism in the case of cortical activity (9).

Normal cortical activity in the cat is influenced only by extremely large, near-fatal amounts of acid or base, as far as can be determined from these records. This would suggest that changes produced by increasing or decreasing the CO_2 tension of the blood are related to the effect of CO_2 itself rather than the resultant change in hydrogen ion concentration of either the blood or tissues. The same has been suggested for the respiratory center; Hooker, Wilson and Connett (11) state that while alkalosis tends to depress the respiratory center and acidosis to stimulate it, blood containing a high tension of carbon dioxide causes greater activity of the respiratory center than another specimen of the same hydrogen ion concentration but with a low carbon dioxide tension. Perhaps it would be preferable to drop the use of "alkalosis" and "acidosis" when referring to such changes.

On the other hand Marshall and Nims (12) assert that the pH at the surface of the cortex does not parallel that of arterial blood following intravenous injection of fixed acids or bases and that hydrochloric acid results only in a momentary period of acidity of the cortex and that sodium bicarbonate produces inconsistent changes in the cortical pH. Gesell and Hertz-

man (7) find this to be true in regard to sodium bicarbonate; they state that increased respiration (as seen in acidosis) commonly occurs following its intravenous administration. However, they express the belief that sodium carbonate (used in our experiments) constantly produces a more alkaline state in the tissues and believe that the respiratory rate is a good index of pH change in the tissues following administration of acids or bases.

In spite of the stability of the electrocorticogram in regard to change in hydrogen ion concentration of the blood, alterations in pattern do occur in the presence of large changes or under additional abnormal conditions, as in insulin hypoglycemia. When they do occur, they appear to depend to a certain extent on the pattern present prior to the injection. "Smooth" waves in general remain smooth (Fig. 4A) and "sharp" waves remain sharp (Fig. 3B). An excess of base tends to mobilize high voltage slow activity and to obliterate low voltage fast activity while acid tends to produce the reverse effect. Dubner and Gerard (4) have described an increase in frequency from the lateral geniculate body following an increase in hydrogen ion concentration, but state that alkalinity (produced by NaOH) abolishes all electrical activity from this region.

Summary

1. The minimal amount of acid (HCl) or alkali (Na_2CO_3) injected intravenously necessary to produce changes in the respiratory rate of the anesthetized cat does not disrupt the cortical potentials.

2. Changes in the electrical activity of the cortex following acidosis or alkalosis occur only if near-fatal amounts are injected or, in the case of alkalosis, if an abnormal pattern has already been established as in insulin hypoglycemia.

3. While intravenous injection of acid causes dilatation of the pial arteries in most cases and intravenous injection of alkali causes constriction in most cases, these changes are not obviously related to the alterations of cortical potentials.

REFERENCES

1. Adrian, E. D., and Moruzzi, G. High frequency discharges from cerebral neurones. *J. Physiol.*, 1939, *95:* 27P–28P.
2. Bremer, F., and Thomas, J. Action de l'anoxémie, de l'hypercapnie, et d'acapnie sur l'activité electrique du cortex cérébral. *C. R. Soc. belge Biol.*, 1936, *122:* 1256.
3. Davis, H., and Wallace, W. McL. Electroencephalographic and subjective changes produced by standardized hyperventilation. *Trans. Amer. neurol. Ass.*, 1941, *67:* 139–143.
4. Dubner, H. H., and Gerard, R. W. Factors controlling brain potentials in the cat. *J. Neurophysiol.*, 1939, *2:* 142–152.
5. Fishberg, A. M. *Hypertension and nephritis.* Philadelphia, Lea & Febiger, 1939 (see p. 248).
6. Gerard. R. W. Factors influencing brain potentials. *Trans. Amer. neurol. Ass.*, 1936, *62:* 55–60.
7. Gesell, R., and Hertzman, A. B. The regulation of respiration. IV. Tissue acidity, blood acidity and pulmonary ventilation. A study of the effects of semipermeability of membranes and the buffering action of tissues with the continuous method of recording changes in acidity. *Amer. J. Physiol.*, 1926, *78:* 610–629.

8. GIBBS, F. A., and GIBBS, E. L. *Atlas of encephalography*. Published by the authors, Cambridge, Mass., 1941.
9. GIBBS, F. A., WILLIAMS, D., and GIBBS, E. L. Modification of the cortical frequency spectrum by changes in CO_2, blood sugar and O_2. *J. Neurophysiol.*, 1940, *3:* 49–58.
10. HEYMANS, C., and BOUCKAERT, J. J. Nouvelle démonstration de la sensibilité réflexogène du ganglion carotidien aux excitants chimiques. *C. R. Soc. Biol., Paris*, 1932, *111:* 654–656.
11. HOOKER, D. R., WILSON, D. W., and CONNETT H. The perfusion of the mammalian medulla: The effect of carbon dioxide and other substances on the respiratory and cardio-vascular centers. *Amer. J. Physiol.*, 1917, *43:* 351–361.
12. MARSHALL, C., and NIMS, L. F. The pH of the cerebral cortex in relation to that of arterial blood. *Yale J. Biol. Med.*, 1940, *13:* 117–121.
13. WOLFF, H. G. The cerebral circulation. *Physiol. Rev.*, 1936, *16:* 545–596.

A DEFICIENCY IN THE PHRENIC RESPIRATORY DISCHARGES PARALLEL TO RETROGRADE DEGENERATION

G. H. ACHESON, E. S. LEE, AND R. S. MORISON
Departments of Physiology and Anatomy, Harvard Medical School, Boston, Massachusetts

(Received for publication March 21, 1942)

THE ANATOMICAL CHANGE occurring in nerve cells after the section of their axons has been extensively studied in the pursuit of neuroanatomical knowledge by the method of Nissl. Little effort has been made, however, to correlate these changes with function. The present study was designed to test whether, in the course of retrograde degeneration, the physiological properties of neurons are altered. As a first approach to the subject, the phrenic was studied.

METHOD

Cats of either sex, weighing from 2 to 5 kg. were used. Under ether anesthesia either phrenic nerve was cut aseptically with a minimum of traction. Two to 200 days later the animal was anesthetized with dial (Ciba, 0.7 cc. per kg. intraperitoneally), a tracheal cannula was inserted, and the phrenics were exposed in the neck. One or more of the roots of each nerve were carefully dissected for a length of 2 to 3 cm. and cut distally. The nearby muscles were retracted and the space so exposed was made into a moist chamber by pads of cotton wet with mammalian Ringer's solution. The distal end of each root was crushed and the nerve was suspended in the air upon a pair of silver wire electrodes (interelectrodal distance 6 to 15 mm.), with the crushed area on the distal electrode for monophasic recording. The region of the nerve from which the record was taken was always at least 10 mm. from the neuroma which resulted from previous section. At this level the two nerves were grossly indistinguishable to the eye.

The respiratory discharges were led through a capacity coupled amplifier to a cathode-ray oscillograph. The coupling condensers were arranged so as to minimize slow shifts in the record and emphasize signals with the time characteristics of axon spikes. The amplified discharges were also passed through a loud speaker. In some instances acoustic analysis was more informative than the photographic record of the cathode ray.

The respiratory discharges in symmetrical roots of the two phrenics were compared. In order to avoid artificial differences in the amplitude of the recorded signal in each experiment the two roots were prepared, as nearly as possible, in the same way, and the conditions of recording were kept constant. Significant differences (greater than 20 per cent) in the amplitude of the records from the two phrenics were found in 3 of the 12 control animals. Hence the results to be presented must be considered quasi-statistically.

At the conclusion of the experiments some of the animals were perfused with chloral hydrate and formamide as recommended by Bank (1). Segments 4, 5 and 6 of the cervical cord were then removed and one of them stained for synaptic end-bulbs by the Davenport (3) modification of Cajal's silver method. Other segments were serially sectioned at 10 to 12μ and alternate sections stained by the Nissl and Bodian methods. The fixative used allowed excellent preparations to be made with all three methods.

RESULTS

In the animals tested 2 to 7 days after section of one phrenic nerve the spontaneous respiratory discharges from the two phrenic nerves were not significantly different. Those examined from the 8th to the 21st day showed a progressive decrease of the amplitude of the discharge recorded from the

previously sectioned phrenic. By the end of the second week this discharge was only about a third of that from the control side (Fig. 1). A week later the change was most marked; in 2 of 3 animals tested after this interval no respiratory discharge could be distinguished from the background noise on the side of the operation, whereas the control phrenic produced discharges of the usual size. After 21 days there was a gradual recovery of the discharge from the previously sectioned nerve, which reached the magnitude of the control between the 40th and 78th days.

The time course of this decreased respiratory discharge following section of one phrenic is charted in Fig. 2. Each rectangle on the graph represents one experiment. The amplitude of the respiratory discharges recorded from the previously sectioned phrenic, expressed as per cent of that of the acutely sectioned, control phrenic, is plotted against days elapsed since the test

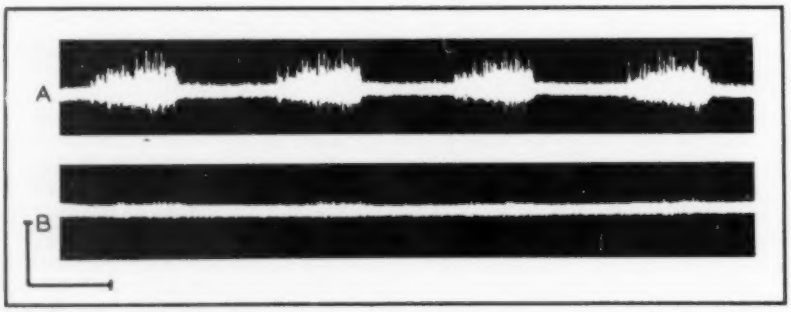

FIG. 1. Deficiency of respiratory discharge recorded from the right phrenic nerve, which had been cut close to the diaphragm 15 days previously. Cathode-ray oscillographic records. The lines at the left and below represent, respectively, 50μV. and 1 sec. A, left, control phrenic. B, right, test phrenic.

phrenic was cut. For this purpose the records were traced in outline through an enlarger and the average maximal height of the asynchronous discharge estimated for several typical breaths. In the 2 experiments in which no respiratory discharge could be detected from the previously sectioned nerve, the noise level of the record was taken as the maximal discharge. The background noise was 8 to 12μV. in favorable cases, and the respiratory discharges on the control side, while usually 30 to 40μV., occasionally reached 200μV.

The level at which the phrenic was sectioned did not affect significantly the course or severity of the change described. In Fig. 2 the solid rectangles represent animals in which the nerve was cut near the diaphragm, and the open rectangles those in which it was cut at the level of the first rib. The decreased respiratory discharge from the previously sectioned nerve occurred as readily on the right as on the left. When present, it was as marked in the 4th as in the 5th or 6th cervical root of the phrenic nerve. The magnitude of the discharge from a given root did not depend on the intactness of the other ipsilateral or contralateral roots.

Two types of experiment were done to show that it is the interruption of

the axons rather than some other factor in the surgical procedure which leads to the deficiency of respiratory discharge. When the chest wall was opened and the lung was retracted, but the phrenic nerves were not molested, the deficiency did not appear. Moreover, when the chest wall was opened on the right and the left phrenic was cut, or vice versa, the deficiency of respiratory discharge always occurred on the side on which the phrenic had been cut.

In several of the animals in which one phrenic had been cut low in the chest, both phrenics were excised after recording the respiratory discharges and studied in a moist chamber at room temperature. The excitability of the two nerves, one of which had been cut peripherally 1 to 3 weeks previously,

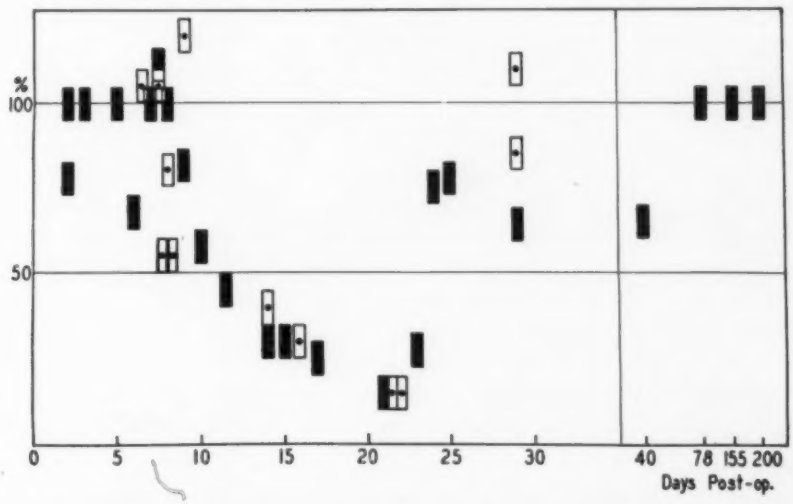

FIG. 2. Chart showing time course of deficiency of respiratory discharge from phrenic nerve following peripheral section. Each rectangle represents a single experiment. Abscissae: days after section of the nerve. Ordinates: amplitude of respiratory discharge from the previously sectioned, test phrenic expressed as per cent of that from the opposite, acutely sectioned, control phrenic. Solid rectangles: test phrenic cut near diaphragm. Open rectangles: test phrenic cut at level of first rib.

was not grossly different. Conduction velocity was usually slower in the previously sectioned nerve. When correction was made for conduction velocity, there was no significant difference in the amplitude of the maximal spike for the A fibers of the two nerves.

Microscopic examinations of the spinal cord in cervical segments 4, 5 and 6 were made in several instances in which the deficiency of respiratory discharge described above was more or less marked (20 to 70 per cent of control). Serial Nissl preparations showed little or no retrograde degeneration in cords from 8 animals in which the phrenic had been cut close to the diaphragm. Bodian and Cajal preparations of these cords also showed little change either in the boutons terminaux or in the neurofibrillae. In one animal

in which the phrenic was cut at the level of the first rib, Nissl preparations showed little or no retrograde changes on the side of the cut nerve.

Discussion

The time course of the deficiency in respiratory discharge described above is strikingly similar to the course of retrograde degeneration in motor neurons, as described by Nicholson (5) and Bucy (2). These authors found relatively mild anatomical changes in the first 8 days following section of motor nerves. From the 9th to the 15th day they describe a marked progressive change in the appearance of the cells, reaching a peak at about 20 days. In the succeeding weeks a gradual return to normal occurs, and by the 40th to 60th day recovery is complete. The physiological change in the phrenic neurons, as tested above by the magnitude of spontaneous discharges, parallels these anatomical changes of retrograde degeneration (Fig. 2). It is interesting to note that Kohnstamm (1899) predicted this relation and suggested that it should be tested on the phrenic nerve.

The fact that the change in function of the neurons follows the same time course as does the anatomical change when the latter is present suggests that a common metabolic process may underlie both. On the other hand, in the present study the phrenic neurons showed little anatomical change (p. 271). Both Kohnstamm (4) and Sano (7), in reporting retrograde degeneration in these neurons, remarked on the mildness of the alterations when compared with what is seen in the nuclei of cranial nerves. Furthermore, the degree of functional loss does not depend on the level of section (p. 271). This result contrasts with the law of retrograde degeneration frequently cited by the older neuro-anatomists, that the farther away from the cell body the axon is cut, the less marked is the anatomical change. These experiments demonstrate that the correlation between the functional and anatomical processes is not strictly quantitative. They further emphasize that for some aspects of the change resulting from section of the axon the functional test is a more sensitive indicator, while, for others, the anatomical method is preferable.

The studies reported above on the excised nerves exclude axonal changes as the cause of the deficiency of respiratory discharge recorded from the previously sectioned phrenic. One can conclude from these data that, short of death of the cell, which sometimes occurs at the peak of retrograde degeneration (6), the conducting mechanism is little affected by the process underlying the other changes. The significance of the decreased conduction velocity remains obscure. The deficiency of respiratory discharge might be due to changes in one or all of the following processes: (i) initiation of nerve impulses in the phrenic neurons; (ii) conduction over the cell body and dendrites of the phrenic neurons; (iii) synaptic phenomena by which the phrenic neurons are excited; and (iv) similar processes in the higher neurons of the ipsilateral respiratory mechanism. The present data do not differentiate among these possibilities.

Summary

After section of the phrenic nerve the respiratory discharges recorded central to the cut undergo a temporary deficiency (Fig. 1) with a time course (Fig. 2) parallel to that described for retrograde degeneration in other neurons. Yet the phrenic cell bodies may show little or no anatomical change even when this deficiency is extreme. The deficiency is not due to failure of conduction in the phrenic axons.

REFERENCES

1. Bank, E. W. A fixing and staining technique for nervous tissue. *Anat. Rec.*, 1940, *76* (Suppl. 1): 82.
2. Bucy, P. C. Studies in degeneration of peripheral nerves. *J. comp. Neurol.*, 1928, *45:* 129–159.
3. Davenport, H. A. Block staining of nervous tissue with silver. III. Pericellular end-bulbs or boutons. *Stain Tech.*, 1933, *8:* 143–147.
4. Kohnstamm, O. Zur Anatomie und Physiologie des Phrenicuskernes. *Fortschr. Med.*, 1898, *16:* 643–653.
   Ueber retrograde Degeneration. *Schmidts Jb.*, 1899, *261:* 253–270.
5. Nicholson, F. M. Morphologic changes in nerve cells following injury to their axons. *Arch. Neurol. Psychiat., Chicago*, 1924, *11:* 680–697.
6. Ranson, S. W. Retrograde degeneration in the spinal nerves. *J. comp. Neurol.*, 1906, *16:* 265–293.
7. Sano, F. Etude sur l'origine réelle du nerf diaphragmatique. *J. méd. Brux.*, 1898, *3:* 501–507.

ORIGIN, CONDUCTION AND TERMINATION OF IMPULSES IN THE DORSAL SPINO-CEREBELLAR TRACT OF CATS

HARRY GRUNDFEST AND BERRY CAMPBELL
Laboratories of The Rockefeller Institute for Medical Research, New York

(Received for publication March 21, 1942)

THE DORSAL SPINO-CEREBELLAR TRACT (Flechsig's tract), which extends from the lumbar cord upward through the restiform body into the cerebellum, presents a favorable object for an electrophysiological study of ascending pathways formed within the cord by secondary neurons. Since it lies just lateral to the line of entry of the dorsal roots and on the surface, the tract is easily accessible to the placement of recording electrodes. It is conspicuous anatomically because a considerable portion of its fibers are large; Häggqvist (7) found that in man about 30 per cent of the myelinated fibers range from 10 to 18μ in diameter. In comparison, only 1 per cent of the fibers of the fasciculus gracilis are larger than 10μ, and of these, furthermore, only a scattered few fall in the range from 11 to 14μ. Flechsig (5), Mott (14), and Van Gehuchten (22), with three different methods established that the larger fibers of Flechsig's tract have cells of origin within Clarke's column (the nucleus dorsalis). Many subsequent workers have confirmed this observation, and also that of Mott (13) that collaterals from the primary sensory fibers of the dorsal funiculus terminate in the vicinity of Clarke's cells. Whether the fibers of Flechsig's tract originate from cells of the ipsilateral, the contralateral, or from both columns has been a subject in question. Discussions of this point are found in Kappers, Huber and Crosby (10), Pass, (15), and Strong, (19). Likewise a matter of differing opinions has been the question of the extent of the distribution of the fibers within the cerebellum (12, 9, 1; and discussions, 10, 11). It is agreed, however, that the fibers of Flechsig's tract end mainly within the cortex of the rostral part of the vermis.

Afferent stimulation of the nerves of the hind limb of the cat, or of the corresponding dorsal roots, initiates electrical activity in Flechsig's tract. This activity has been recorded with electrodes made from steel needles 50 to 250μ diameter in the shank and insulated except for the tip, which is especially sharpened to minimize gross damage to the spinal cord. The most satisfactory electrodes at the present time are 250μ needles ground on a long taper for about 8 mm. and then pointed with a sharp taper to a tip of 5 to 10μ. Needle electrodes so prepared combine mechanical rigidity and ease in insulating with a minimum of destructiveness to the tissue of the spinal cord. The recording and stimulating arrangements used in these experiments are those standard for the laboratory.

The Potentials in Flechsig's Tract

Distribution of potential. The potential recorded from the tract is sharply localized in the dorsal part of the lateral funiculus of the cord, and it is recorded either when the active needle electrode is on the surface of the region or in its most superficial stratum. Often a number of trials have been necessary in placing the needle electrode before the maximum potential could be observed. This is probably correlated with the dispersed nature of the tract as observed in the successive degeneration experiments of Sherrington and Laslett (18) and by MacNalty and Horsley (12). The impulses can be followed from the midlumbar level of the spinal cord into the white matter of the cerebellum.

Form of the potential. The electrical activity set up in the tract by a single afferent volley is recorded as a positive potential (Fig. 1, *a* to *e*) which reaches a maximum in 2 to 5 msec. and lasts usually less than 10 msec. In comparison, the potential similarly set up at the same level in the dorsal column is much larger and longer. At the periphery of the dorsal spinocerebellar tract, or in its center if weak stimuli are used, the response often is a series of positive spikes of 0.4 to 0.6 msec. duration (Fig. 7 to 12). These spikes are monophasic and probably represent leads from a few synchronously active fibers.

Axonal properties of spike responses. It cannot be said with certainty which, if any, of the spikes of Fig. 7 to 12 represent activity of individual cells. In some instances the composite nature of the response can be ascertained from the variations in amplitude which it exhibits, but the short duration of these spikes, from 0.4 to 0.6 msec., indicates that the tract axon produces an electrical response which has the same duration as do axonal spikes of peripheral nerve (6).

Effectiveness of various afferent stimuli in producing potentials in the tract. Potentials of similar magnitude and time relations are evoked in the tract by stimulation of the ipsilateral tibial nerve, peroneal nerve, or a dorsal root; and smaller responses are evoked by stimulation of nerves to individual muscles. No impulses are evoked on stimulation of the saphenous nerve, but activity attributable to propriospinal fibers can be recorded in the tract region proximal to the entry zone of the saphenous nerve fibers. Likewise, no impulses are evoked in the tract by stimulation of the contralateral tibial nerve. Flechsig's tract is, therefore, set into activity by fibers afferent for muscle proprioceptors and is uncrossed, but a small crossed component is not ruled out. The evidence for the above experimental findings is shown in Fig. 1.

The distribution of the potentials in Flechsig's tract in response to stimulation of either the tibial or the peroneal nerve appears to be essentially the same (Fig. 1, *a*, *b*). Later, on the basis of the interaction of the responses evoked by the two nerves, it will be shown that there is considerable overlap between the synaptic terminations of the afferent fibers of various nerves on the cells of Clarke's column. The absence of sharp localization in the tract may in part be caused by this overlap.

Conduction of impulses in Flechsig's tract. Figure 2 makes a comparison of the temporal course of the activity in Flechsig's tract with the potential

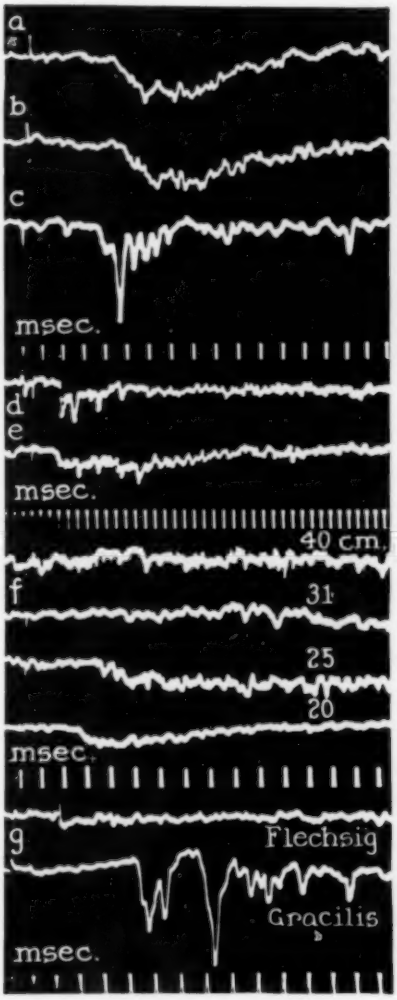

FIG. 1. Potentials recorded from the dorsal spino-cerebellar tract of the cat, in response to stimulation of the following ipsilateral afferent fibers: *a*, the tibial, and *b*, the peroneal nerve; *c*, dorsal root, SI; *d*, hamstring nerve; *e*, nerve to quadriceps femoris muscle; *f*, saphenous nerve at the four distances of conduction indicated in the figure. In *g*, the upper record is from the tract on stimulation of the contralateral tibial nerve and the lower record from the dorsal column ipsilateral for the nerve, both taken at the same level in the cord.

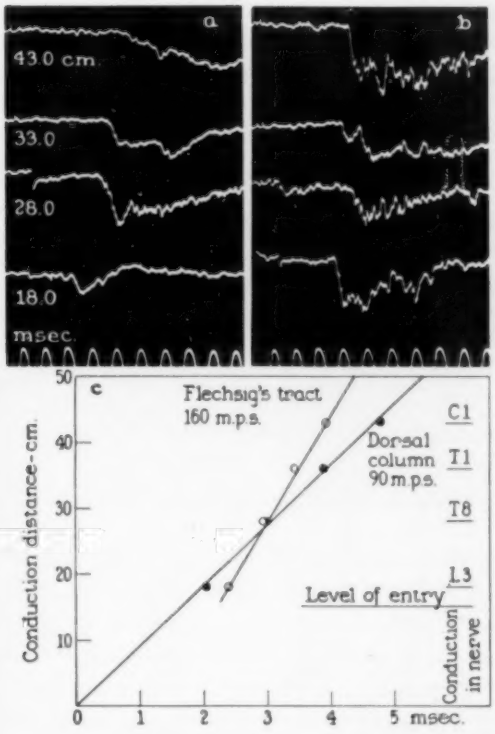

FIG. 2. The temporal course of impulses ascending in the dorsal column and in Flechsig's tract in response to tibial stimulation. *a*, responses in the dorsal column; *b*, responses in the tract at the four conduction distances given on the records. The graphic formulation of the data is given in *c*. ● . . . dorsal column; ○ . . . Flechsig's tract. The ordinates on the left give the distances of conduction; on the right are indicated the approximate levels in the spinal cord.

developed in the dorsal columns as the two sets of impulses ascend in the cord following a stimulus to the ipsilateral tibial nerve. The responses were recorded in both tracts at four distances of conduction (Fig. 2, *a*, *b*). The times at which the earliest response arose in each tract are plotted against the con-

duction distance from the point of stimulation in Fig. 2 *c*. The slopes of the straight lines which join all the points of each set represent velocities with which the impulses are conducted in the two tracts. In the experiment illustrated the conduction velocity is 160 m.p.s. for Flechsig's tract, while in the dorsal column it is nearly half, 90 m.p.s. That impulses are conducted more rapidly in fibers of Flechsig's tract than in the primary afferent neurons

Table 1. Summary of 11 experiments on velocity of conduction of impulses in Flechsig's tract and in dorsal column.

Date	Stimuli applied to:	Rectal temperature (°C.)	No. of points in measurement	Largest conduction distance (cm.)	Conduction velocity (m.p.s.)		Ratio CV Flechsig's tract / CV dorsal column
					Flechsig's tract	Dorsal column	
Dec. 12, '40	Tibial nerve	37.0	4	28	110	56	1.98
Jan. 6, '41	" "	39.5	4	43	160	90	1.78
" 7, '41	" "	37.5	2	43	120	78	1.54
" 21, '41	" "	38.7	5	44	130	75	1.73
" 23, '41	" "	40.0	5	45	125	60	2.10
" 27, '41	" "	37.3	2	24	110	59	1.86
" 28, '41	" "	39.1	6	43	85	50	1.70
" 28, '41	Tracts	39.1	3	21	95	50	1.90
Feb. 5, '41	"	38.0	2	17	103.5	77.5	1.33
" 7, '41	"	39.8	2	16	155	72.5	2.10
" 27, '41	Tibial nerve	37.0	2	38.5	125	64.0	1.95

of the dorsal column was observed in eight experiments, the results of which are summarized in Table I.

The experiments on which are based the data of Fig. 2 and of Table I required some precautions in technique which deserve to be mentioned. The animals were maintained at temperatures within the range of physiological normality. These are enumerated for each experiment in Table I. To avoid cooling the portions of the spinal cord over which the conduction velocity was being measured, the first exposures were always made at the highest levels from which recordings were being taken. The subsequent exposures at lower levels were made in order down the spinal column.

A second method for determining the velocity of conduction was employed in three experiments (Fig. 3, Table I). The dorsal column or Flechsig's tract was stimulated directly at higher levels of the cord and records were made in the lower thoracic and the lumbar cord. Controls showed that the stimulus, when applied to the one tract by means of paired needle electrodes insulated except at the tip, did not spread to the other tract. The velocities of conduction in Flechsig's tract and in the dorsal column obtained in these experiments were entirely concordant with the measurements of the series first cited.

Returning to the records of Fig. 2, it is seen that in the lumbar cord activ-

ity is initiated in the dorsal column before the beginning of activity in Flechsig's tract. At the level of the seventh to the ninth thoracic segments the more rapidly progressing impulses in Flechsig's tract catch up with the activity ascending in the column and then forge ahead to arrive at the upper cervical levels of the cord about one millisecond before the latter. In the course of synaptic transmission in the funicular nuclei, secondary neurons activated by impulses afferent in the columns are further delayed (20). The earliest activity in the medulla is thus a product of the impulses brought to it in Flechsig's tract and not by those carried in the dorsal columns. The

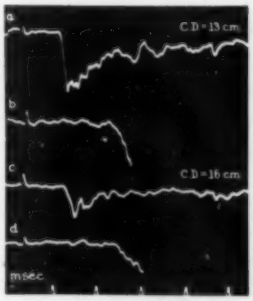

FIG. 3. Comparison between the velocities of conduction of impulses descending in Flechsig's tract (*a*, *c*) and in the fasciculus gracilis (*b*, *d*) in response to direct stimulation of the two tracts at T3. The recording electrode was in L2 for the upper records (C.D. = 13 cm.) and at L4 (C.D. = 16 cm.) in the lower set. The velocities determined from these records are 155 m.p.s., for the fibers of Flechsig's tract and 72.5 mp.s. for those of the dorsal column.

impulses which reach the medulla and the cerebellum via the fibers of Flechsig's tract will be discussed in a later section of this paper.

Relation between diameter of intraspinal fibers and their velocities of conduction. The greater velocities of conduction of the fibers of Flechsig's tract, in comparison with the rates at which impulses are conducted in the dorsal columns, appear to be correlated with the larger size of the fibers of Flechsig's tract. Häggqvist (7) found that 1 per cent of the fibers of Flechsig's tract are 16 to 18μ in diameter. In the dorsal column, on the other hand, while there are some fibers of 12 to 14μ diameter, the group having diameters of 10 to 11μ is the first to amount to 1 per cent of the total number. The ratio of the diameters of the means of the two groups is 17:10.5, or 1.6. The average ratio of the velocities with which impulses are conducted in the fibers of the two tracts is 1.8 (Table I). It appears likely, then, that the velocity with which impulses are conducted within the spinal cord bears the same relation to the diameters of the fibers as that which has been found in the peripheral nervous system (8, 6).

Lower extent of tract. Unambiguous evidence of the presence of Flechsig's tract in the lower lumbar cord is found in the experiments in which the tract was stimulated directly. Figure 3 shows the record of activity in the dorsolateral column of lumbar segment 4 in response to stimulation of Flechsig's tract in the upper thoracic region of the spinal cord. The origin of the tract must, therefore, lie at or below the fourth lumbar level.

In other experiments, responses were observed only at or above the third or the second lumbar segments. This variability is to be expected in view of the known anatomical variability of the level at which Clarke's column is

formed. Beck (1), in degeneration experiments, identified the tract in the fifth lumbar segment.

Origin of Impulses in Flechsig's Tract

Synaptic delay in Clarke's column. Since Flechsig's tract is composed of second order neurons, which have their cells of origin in Clarke's column, a delay is to be expected between the arrival of activity at the collaterals of the afferent primary neurons and the onset of post-synaptic activity in Clarke's cells. This delay appears most probably to account for the interval between the appearance of the response in the dorsal column and in Flechsig's tract which is shown in the records of Fig. 2 *a* and *b*. The synaptic delay can be measured from the graph in Fig. 2 *c*, on the assumption that in the animal of this experiment the origin of the tract occurred at the fourth lumbar segment, as is indicated in the figure. The delay so measured is 0.55 msec.

Another method has also been employed to measure the magnitude of the synaptic delay (Fig. 4). In the lumbar and lowest thoracic levels of the cord, the large potential which represents the activity of Flechsig's tract is preceded by a smaller potential which begins synchronously with the onset of activity in the dorsal column at the same level. It is interpreted by us as the direct pick-up of the potential from collaterals of the fibers of the dorsal column to the region of Clarke's column. The interval between the start of this small potential and the tract response is then the interval of the synaptic delay at Clarke's column. The synaptic delay measured from the records of Fig. 4 ranges from 0.5 to 0.9 msec.

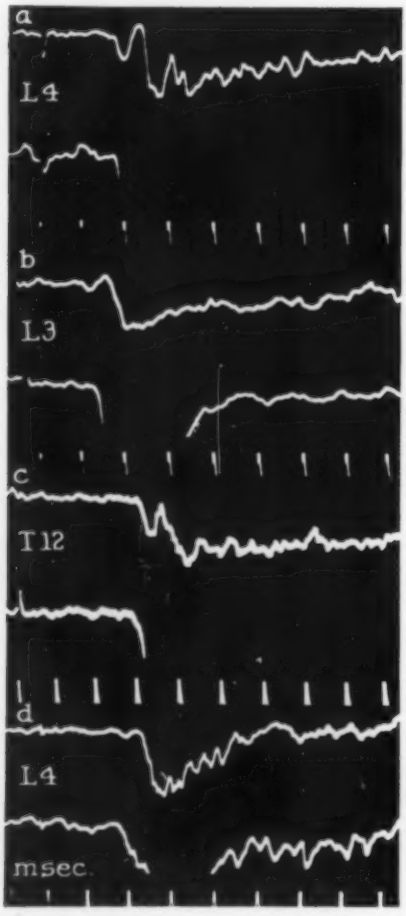

Fig. 4. Activity recorded in Flechsig's tract (upper record of each set) and in the dorsal column (the corresponding lower record) on stimulation of the ipsilateral tibial nerve. The recording electrode was in the lowest thoracic or in the lumbar levels of the spinal cord, as indicated on the records. The response occurred earlier in the dorsal column than in Flechsig's tract by 0.5 to 0.9 msec. Ahead of the tract response is seen a small deflection in the tempo of the onset of activity in the column.

Synaptology of Clarke's Column

The degree of complexity in the synpatic connections of Clarke's cells is at present unknown in terms of anatomical data. Some indication of their activity is, however, obtainable on the basis of the electrical records. In almost every experiment it has been found that the micro-electrode can be so placed in the tract as to record a series of discrete spikes. When such spikes are recorded, their number can often be changed by varying the

Fig. 5. Response in Flechsig's tract to progressively stronger stimulation of the tibial nerve. At the strength of stimulation used in *a*, no response was elicited. With somewhat stronger stimuli, the first response was small, *b*. This increased with stronger stimuli, *c*, but thereafter remained essentially unchanged in later records with still stronger stimulation of the nerve. Another group of elements, recorded as a sharp spike, made its appearance in *c*. With stronger stimuli to the nerve, the first spike response arose earlier and was followed by more spikes and other, less well-defined activity, *d* to *h*.

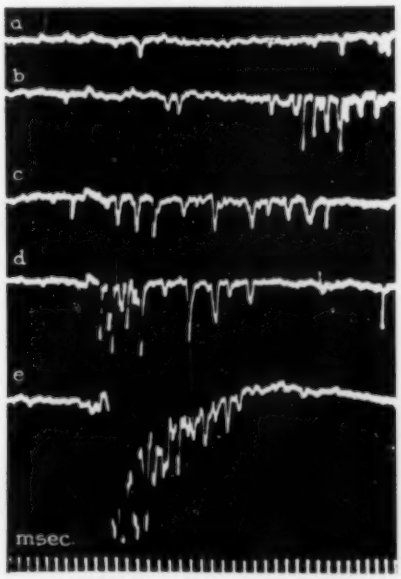

Fig. 6. Another experiment showing the modification of the responses in the tract by progressively increasing strength of stimulation of the tibial nerve. *a*, *b*, very weak stimuli, *b* the stronger. The late activity in *b* may be spontaneous in origin, but is in the pattern of the response to stimulation in the subsequent records. *c*, *d*, further increases in the strength of the stimulus. With the stronger, *d*, the multiple response arose earlier, was larger, and the spikes were more closely grouped. Response to supramaximal stimulation of the nerve is shown in *e*.

strength of the afferent stimulus. A weak stimulus to the nerve produces one or a few spikes, and these increase in number and in frequency progressively as the stimulus is strengthened (Fig. 5). The interval between the stimulus and the first appearance of the spikes may also be decreased as the stimulus is increased (Fig. 6). *These several observations indicate that there is no one-to-one relationship between fibers of the dorsal column and Clarke's cells, that sensory fibers of different electrical thresholds activate the cells, and that there is probably a multiple order of synpatic activation of the cells.*

Interaction on the responses in Flechsig's tract between the effects of stimulating different nerves gives more specific information on these findings. This is particularly the case in experiments in which the activity elicited under the recording micro-electrode takes the form of discrete, identifiable spike potentials.

Convergence of excitation from afferent fibers. In the experiments of Fig. 7, a stimulus to the peroneal nerve caused in the tract a typical response in the form of three spikes, each lasting 0.5 to 0.6 msec. (records *a*, *b*, *c*). The form and amplitude of the three remained remarkably constant throughout the experiment and persisted through several small displacements of the micro-electrode. This last in itself indicates that the potentials were being conducted in a group of homogeneous fibers which had been nearly uniformly activated by a pool of cells in Clarke's column. The time of arrival of the first spike remained constant throughout the experiment at 2.4 msec. after the shock. The second, smaller spike followed the first by a constant interval of 0.9 msec. The third spike, in amplitude equal to or greater than the first spike, followed the first by an interval of 4.2 msec. in the majority of the records of the figure. This interval varied considerably, becoming shortened to 4.0 msec. (records *a* and *q*), and in two records (*c* and *t*), it was diminished by 0.6 msec.

Only a single spike was elicited on stimulating the hamstring nerve. This arrived at the electrode after the same interval as did the first response to stimulation of the peroneal nerve. In form the two spikes were also closely similar, but the response to the hamstring nerve could be equal to, or greater or smaller than the first response to the peroneal nerve (records *d*, *k*, *r*). This observation indicates that the spike in response to stimulation of the hamstring nerve was also the product of the simultaneous activation of a pool of Clarke's cells, and that the impulses were conducted in the tract in a homogeneous group of fibers.

The two early spikes, caused by the stimulation of either nerve, were due to the activation of the same pool of Clarke's cells by the collaterals of fibers afferent in either of the two nerves. This is shown by the results of applying both stimuli at intervals varying from 1.2 to 1.8 msec. (records *e* to *i*). The single spike caused by stimulation of the hamstring nerve was completely inhibited when there was activity at the recording electrode due to the first "peroneal" spike and for well over a millisecond later. The response was partially inhibited at later intervals (records *l*, *m*). The available data do

not permit identification of the third spike of the peroneal response with the activity of the same pool of cells. Inspection of records *p* to *u* indicates, however, that this is probable.

Number of steps involved in the response. The time relations of the triple spike response to peroneal stimulation (Fig. 7) yield information on the

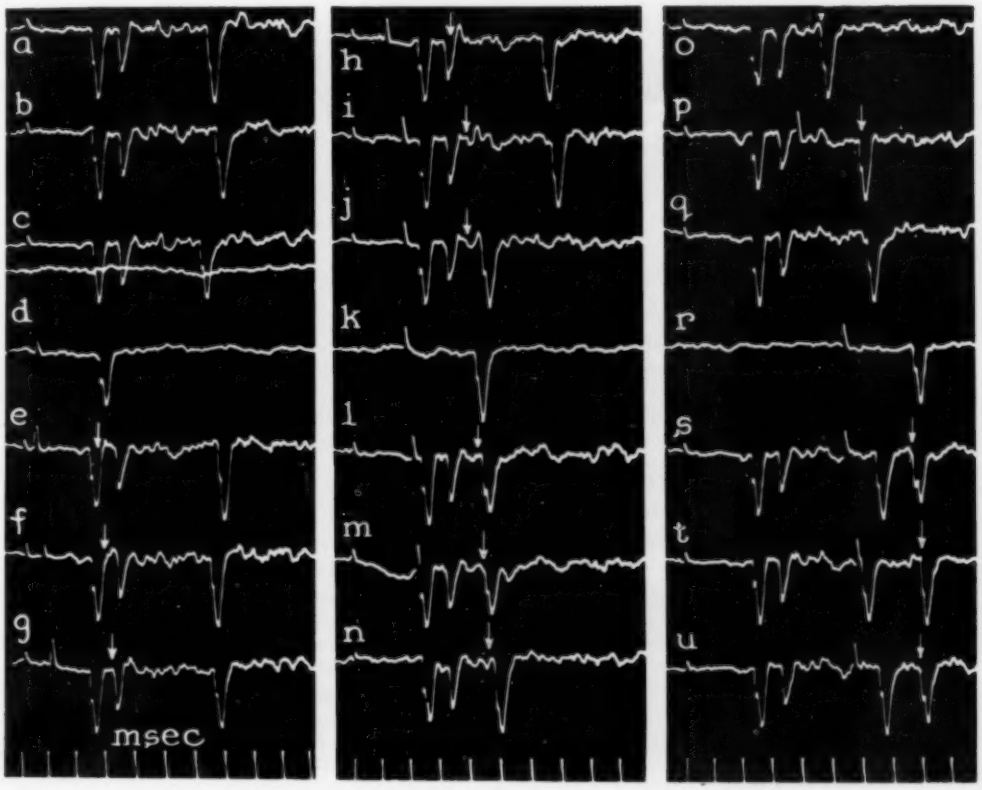

FIG. 7. Interaction between the responses evoked in the dorsal spino-cerebellar tract by stimulation of the peroneal and the hamstring nerves. The response to stimulating the peroneal nerve alone is seen in *a*, *b*, *c*, *q*; that evoked by stimulation of the hamstring nerve alone is shown in records *d*, *k*, *r*. Arrows indicate the expected time of onset of the test response. (Further description of the records is in the text.)

activity which may be involved in the response of Clarke's cells. The first spike in response to peroneal stimulation and the single spike caused by a stimulus to the hamstring nerve represent activation of the Clarke's cells by the direct collaterals of the primary sensory fibers afferent in the two nerves. The responses occurred 2.4 msec. after the stimuli, or 0.6 msec.—a synpatic delay—after the afferent volley had reached the dorsal column at the same level of the spinal cord. The second of the spikes in the response to peroneal stimulation, which occurred 0.9 msec. after the beginning of the first spike, may have been the product of activation of Clarke's cells through

an intercalated neuron, through direct impulses in collaterals of slowly conducting afferent fibers, or through the combined action of impulses reaching the cells from both sources. The third spike of the response most probably was the product of internuncial activity. It arose late, when most of the

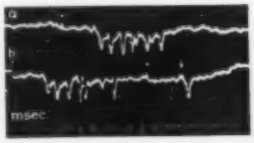

FIG. 8. Inhibition of responses which are mediated by internuncial activity. A single stimulus to the tibial nerve produced a series of spike responses in the tract, *a*. All but the first of these was inhibited by the conditioning effect of activity initiated through another stimulus to the tibial nerve 11 msec. earlier, *b*. The stimulus artifacts are recorded by dots.

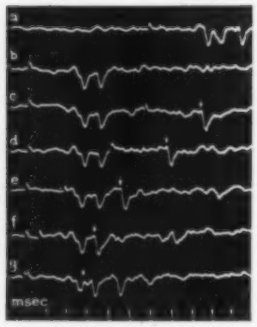

FIG. 10. Responses of Clarke's cells to internuncial activity set up by summation of stimuli to different nerves. *a*, the response to stimulation of the tibial nerve, and *h* to stimulation of the peroneal nerve. In records *b* to *g* and *i* to *n*, both stimuli were applied together, the tibial stimulus leading in the records of the left column, and the peroneal stimulus leading in the column on the right. The extra responses of records *b* to *d* and *i* to *k* were set up by internuncial activity initiated by the summation of excitation via the two afferent volleys. The time at which the onset of the response to the second stimulus was to be expected is marked with an arrow. (Further description of the records is in the text.)

FIG. 9. Differential effects of antecedent conditioning activity on the primary and internuncial activation of Clarke's cells. Same preparation as for the preceding figure, but with the conditioning and testing responses produced by stimuli applied to different nerves. *a*, response in the tract to stimulation of the tibial nerve. *b*, the response, at the same locus, to stimulation of the peroneal nerve. *c*, *d*, *e*, the "tibial" response preceded by conditioning "peroneal" activity. Only the first spike of the test response survived. *f*, this spike fell into the refractory period produced by the second spike of the peroneal response. *g*, the two stimuli were applied within 0.2 msec. of each other. An extra response occurred in the tempo of the third spike of the tibial response of record *a*. The arrows indicate the expected time of onset of the first spike of the test response.

activity in the primary volley had subsided. The stimulus-response time varied by a small amount (0.2 msec.) in the majority of the records, a variation which may be ascribed to the known variability of the synpatic delay. A larger variation (0.6 msec.; records *c* and *t*) probably represents a shortening of the internuncial chain by one link.

Inhibition of responses which involve interneurons. When the "hamstring" spike was able to develop ahead of the third "peroneal" spike (Fig. 7; records *j* to *p*), the latter was inhibited. The inhibition may have been produced by one of two causes. If the third "peroneal" spike were the result of activation of the same group of Clarke's cells, the "hamstring" spike had left these cells in a state of refractoriness too great to be overcome by the activity of the interneurons which mediated the later spike. It is also possible that the collaterals of the fibers afferent in the hamstring nerve, while unable to initiate a pattern of internuncial activity which would lead to the stimulation of the Clarke's cells, nevertheless were able to break up the pattern initiated by the collaterals of the peroneal afferents, and thus to inhibit the late "peroneal" spike. Whatever the cause, activity in Flechsig's tract which is mediated by interneurons is readily inhibited.

Activity which is set up in Clarke's cells through internuncial chains may be inhibited for a considerable time. Eleven msec. after a stimulus to the tibial nerve had set up a volley of spike activity in the tract (Fig. 8 *b*) a second stimulus to the tibial nerve elicited only the first of the series of spikes, that caused by the activation of the Clarke's cells by direct collaterals from the primary neurons.

Inhibition of activity mediated by interneurons occurs also when the internuncial activity is initiated through volleys in different afferent paths. In the experiment of Fig. 9, stimulation of the tibial nerve caused a triple spike response in the tract (*a*). Stimulation of the peroneal nerve caused only two spikes (*b*). On conditioning the tibial response with antecedent peroneal activity, the last two spikes of the tibial response were inhibited completely (*c* to *e*). There remained only the first spike, that due to the stimulation of the Clarke's cells by the collaterals of the primary afferent fibers. This spike also was absent when its onset fell into the refractory period set up by the antecedent responses to peroneal stimulation (*f*, *g*), showing that the same elements were active in response to stimulation of either nerve.

Excitation by summation of stimuli. Figure 10 shows an experiment in which stimulation of two nerves within the summation interval evoked an extra response in the tract.

The response to stimulation of the tibial nerve (record *a*) or of the peroneal nerve (record *h*) was in this experiment a compound of several spike responses. It was longer than the minimal duration observed in other experiments, and appeared with a double peak of positivity, sometimes nearly fused and sometimes more distinct. Two components were clearly seen when the response was evoked by stimulation of one nerve against a background of conditioning activity set up via the other nerve (records *g*, *n*). The stimulus-response interval differed for the two nerves, being 3.4 msec. for tibial (*a*) and 2.95 msec.

for peroneal (*h*) stimulation. This difference may have been due to difference in the conduction time in the primary neurons and in the synaptic delay. It served to determine the origin of the response when the two stimuli were pitted one against the other.

Stimulation of both nerves so as to cause the evocation of both responses simultaneously (the peroneal stimulus lagging by 0.45 msec.) caused a combined response (*b*), the early part of which was no larger than that produced on stimulation of the tibial nerve alone. The activity set up by stimulation of either nerve was, therefore, in a common group of Clarke's cells. A de-

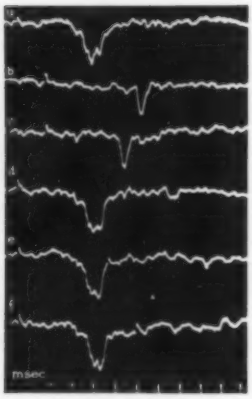

FIG. 11. Responses set up by internuncial activity. *a*, response in Flechsig's tract to stimulation of the tibial nerve. The stimulus-response time was 2.6 msec., indicating direct stimulation of Clarke's cells by collaterals of the primary afferent neurons. *b* and *c*, successive responses to stimulation of the peroneal nerve. These occurred 4.25 and 3.3 msec. after the stimulus and involved internuncial activity of variable degrees of complexity. The subsequent records show complete inhibition of the response evoked through internuncial stimulation by conditioning via the tibial nerve.

layed, additional response was also set up 0.75 to 0.85 msec. after the beginning of the first response. The time of onset of the extra response remained constant within this range when the two stimuli were separated by a considerable interval, whether the tibial stimulus was leading (*c* to *e*) or the peroneal (*i* to *l*). The extra spike was the response of Clarke's cells to stimulation of interneurons which were brought into play by the combined effects of two primary volleys when these were separated by no more than the summation interval. The summation interval measured from this experiment is 0.5 msec. When the interval between the stimuli was greater than the summation interval, the responses were to synaptic stimulation by the direct collaterals of the primary neurons. At first, only the early group of Clarke's cells recovered sufficiently to respond a second time (*f*, *m*); then the late group also recovered (*g*, *n*).

In this experiment it was not possible to ascertain whether the response set up by the interneurons activated within the summation interval represented the activity of the group of Clarke's cells stimulated by the primary neurons, or whether it was due to stimulation of another, previously inactive group. The experiment of Fig. 11, however, shows clearly that cells which are not activated by direct collaterals can be set into activity through internuncial chains of varying length.

In this experiment, stimulation of the peroneal nerve caused only a spike mediated by internuncial activity. This can be ascertained by comparing the stimulus-response time for the tibial response (*a*, 2.6 msec.) with the time for the spikes evoked via the peroneal nerve

(4.25 msec., *b*; and 3.3 msec., *c*). The response of record *b* probably involved two interneurons, while in *c* the chain was shortened to the participation of only one intereuron. As in other experiments described earlier, these responses, which involve internuncial activity, were completely inhibited by antecedent stimulation of the tibial nerve (*d* to *f*).

The foregoing series of experiments indicates that Clarke's cells can be activated by collaterals from a number of primary neurons and by internuncial chains of varying length, through which the impulses from a number of primary pathways converge. Cells not stimulated by one set of primary impulses and the interneurons activated by these may be brought into activity by summation with another set; and conversely, the responses of cells that would be stimulated by one set may be inhibited by another set.

Independence in the activity of other cells of Clarke's column is shown in the records of Fig. 12. Stimulation of the tibial nerve (*a*) and of the peroneal nerve (*b*) produced responses of different forms at the recording electrodes. Combination of the two responses resulted in their addition (*c*, *d*, and *e*).

FIG. 12. Independent activity in Clarke's cells in response to stimulation of two nerves. *a*, tibial, *b*, peroneal nerves stimulated individually. *c*, *d*, *e*, both nerves stimulated in rapid succession.

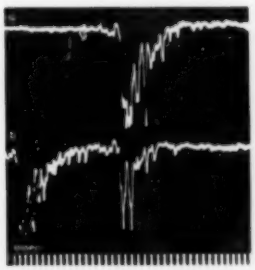

FIG. 13. The effect of previous activity on the large tract response. *a*, the response in the tract to a single stimulus to the tibial nerve. *b*, the second of two responses is diminished in size and duration.

The maximal response. The information obtained under the special conditions where discrete or identifiable groups of responses were recorded serves also for an understanding of the behavior of the large composite response usually recorded in the tract. Since repetitive activity can occur at rates above 500 per sec. (Fig. 7, 10) the response to a maximal afferent stimulus is little affected in its early portion when it is preceded by previous conditioning activity initiated through the same nerve (Fig. 13). Later activity, on the other hand, is considerably reduced. At least in part this may be caused by the inhibition of responses mediated by interneurons. It is also probable that some of the later responses to a single afferent volley are produced by the restimulation of Clarke's cells when the primary afferent neurons are reactivated in the phenomenon which produces the dorsal root reflex discharge (21). Since the reactivation of the dorsal root fibers is easily inhibited by previous activity, this repetition of primary activity will be absent in the

second afferent bombardment of Clarke's cells and will result in the absence of responses in the tract.

The foregoing formulation of the synaptology of Clarke's column is based on electrophysiological data. It calls for internuncial chains of some complexity. No anatomical evidence either for or against the existence of such chains to Clarke's cells is available. The profuse fibrillar network which surrounds the cells is usually considered as formed by collaterals from the primary afferent fibers (23, 2). However, Schäfer (16) described degenerating fibers among the cells of Clarke's column subsequent to lesions of pyramidal fibers. Unfortunately, only the brief statement of this observation was published at a date when the importance of the propriospinal neurons was not yet appreciated (17).

Termination of Flechsig's Tract

Potentials in upper levels of tract. On their course to their final destination in the cerebellar cortex the impulses which ascend in the tract can be observed at the level of the obex (Fig. 14 *b*), in the restiform body (*c*), and in the white matter of the cerebellum (*d*). Activity in the cerebellum starts more than one millisecond before the time at which impulses in the dorsal column (*a*) arrive at the level of the obex.

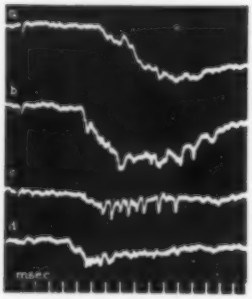

Fig. 14. Potentials in response to stimulation of the ipsilateral tibial nerve recorded from: *a*, fasciculus gracilis, and *b*, Flechsig's tract, at Cl; *c*, in the restiform body, and *d*, in the white matter of the cerebellum. In *d*, the stimulus artifact is at the beginning of the record.

Distribution of fibers in cerebellar cortex. The distribution of the dorsal spino-cerebellar fibers within the cortex of the cerebellum has been obtained by mapping the electrical responses recorded at various points of the cerebellar surface when Flechsig's tract is set into activity. In the experiments most satisfactory for this purpose, the tract was stimulated directly in the lower thoracic region of the spinal cord. On the direct stimulation of the tract, responses were recorded from the rostral portion of the vermis after a short conduction time, which in the various experiments corresponded to velocities of conduction of 100 to 140 m.p.s. These are in the range of the velocities for the conduction of impulses in Flechsig's tract which have been measured in other types of experiments and described in a foregoing section.

The mapping of the cerebellar surface has been most extensively carried out on the dorsal and rostral faces of the vermis. The early potential which corresponds to the direct activity of tract fibers is confined to the vermis and is largest in the lobulus centralis and in the immediately adjacent folia of the culmen. There is relatively little modification in the amplitude of the potential on shifting from the midline to the ipsilateral border of a folium.

Somewhat smaller, but nevertheless definite, potentials are also recorded from the contralateral halves of the folia, but they do not extend so far laterally as do the ipsilateral potentials. The distribution of dorsal spinocerebellar fibers in the cerebellar cortex of the vermis found in the mapping experiments is shown by the shading in the diagram of Fig. 15.

In these experiments less extensive but equally definitive data were obtained which show that few, if any, fibers of the tract go to the declive, or to the pyramis of the vermis, and that none goes to the cerebellar hemispheres.

The cortical distribution of the dorsal spino-cerebellar fibers indicated by these electro-physiological mapping experiments confirms in the main that given by MacNalty and Horsley, Ingvar, and Beck. Our data indicate that fewer of the fibers are distributed to the culmen than was believed by these authors, and none or only a few to other portions of the vermis, in which degenerating fibers have been seen after sectioning the tract. Beck stated that the majority of the fibers fan out laterally and that few or none crossed the midline. The electrical records show that a considerable number of fibers from Flechsig's tract arrive at the midline and that of these a number sufficient to produce recordable activity cross to the opposite side.

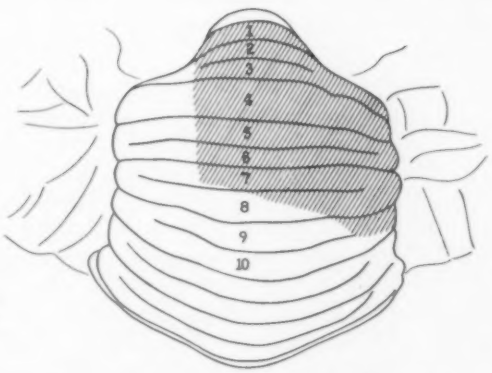

FIG. 15. Diagram showing the bilateral distribution of fibers from the right Flechsig's tract in the lobulus centralis and nearby folia of the culmen of the vermis. The shading represents the region to which fibers are distributed as found from electrical mapping experiments. The numbering of the folia is arbitrary, No. 1 being the first observed above the floor of the fourth ventricle.

Potentials from cerebellar cortex. Considerable exploration has been made of the cortex of the cerebellum in order to observe the electrical response in it resulting from the afferent stimulation of the nerves of the hind limb. The details of these potentials are the subject of continuing investigation and will be omitted here, except in so far as pertains to information on the cerebellar effect of the impulses carried in Flechsig's tract.

In response to afferent stimulation of the tibial or peroneal nerve, the activity recorded from the surface of the ipsilateral rostral portion of the vermis has the simple form shown in Fig. 16. It is essentially identical with the potentials described by Dow (3). The activity is a prolonged positivity which is clearly made up of three components. Other, later components are also demonstrable, but here attention will be centered only on the first three.

The earliest part of the electrical activity occurs 4.0 to 6.0 msec. after the stimulus in the various preparations. Following it there is a second potential at 8 to 10 msec. The dominant potential of the surface electrical re-

sponse is usually the third, which begins at 13 to 17 msec. after the stimulus. This is the potential best seen in the figures of Dow's paper, in which the

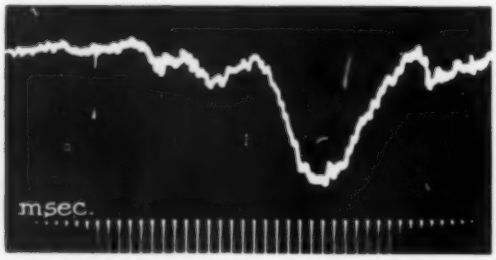

FIG. 16. The electrical activity of the cerebellum in response to stimulation of the ipsilateral tibial nerve, recorded with a needle electrode just touching the surface of the cortex. Three components are clearly seen in the electropositive response and other, later components are also indicated. Further description is in the text.

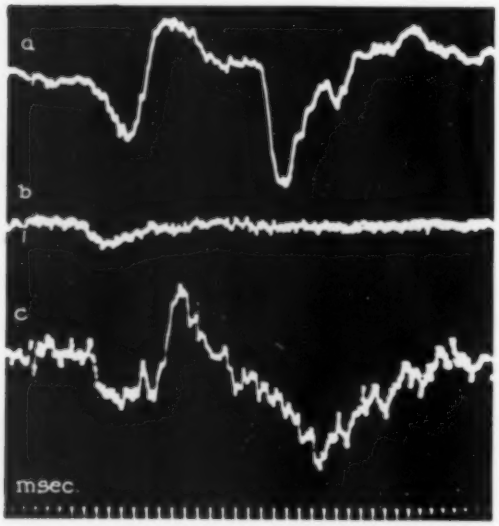

FIG. 17. Modification of the cerebellar response to afferent stimulation, *a*, by asphyxia, *b*, and its restoration following recovery from asphyxia, *c*. Recording needle electrode on the lobulus centralis of the vermis. In the control, *a*, the second potential is negative and the later potentials are positive. During asphyxia, *b*, only the first component of the normal response remains. Upon recovery from early asphyxia, the later components return, *c*. In this record, the second potential begins positive and ends from a negative level.

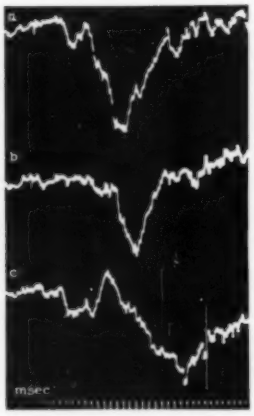

FIG. 18. Cerebellar potentials recorded from the surface of the lobulus centralis of the vermis to stimulation of the ipsilateral tibial nerve *a*. The first positive deflection begins at 4.6 msec. and is succeeded, at 9.7 msec., by a second potential which in this case is also positive. At 13 msec. another large potential is added to the second. Only this last potential remains after Flechsig's tract has been sectioned at T9, *b*. The cerebellar potential of the opposite side in response to stimulation of the other tibial nerve is shown in *c*. All the components of the normal cerebellar potentials are present as in *a*. The second potential component is smaller than in *a*. Its initial positivity goes over into negativity. The third potential is large and is followed by still later components.

records were made at a slow rate of traverse of the electron beam. When the recording needle electrode penetrates the surface of the cortex, the observed response undergoes numerous modifications. These modifications are to be borne in mind when Fig. 16 to 20 are examined. While the magnitudes and signs of the components of the potentials vary in these records, the basic pattern is nevertheless evident.

The first component of the cerebellar potential, as is indicated from its early time of onset, represents the direct activity of the fibers afferent in Flechsig's tract. Further proof of this has been obtained in two ways. Ischemia of the cerebellum, or the early stage of asphyxia of the animal, abolishes all but the first component of the cerebellar potential. This persists long after the disappearance of the later components (Fig. 17, *b*). On

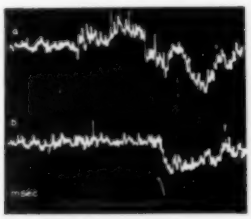

FIG. 19. Cerebellar activity evoked at one locus on stimulating different afferent nerves. At this locus, the first two components of the activity produced by stimulating the ipsilateral tibial nerve, *a*, are negative. Only late potentials of cerebellar response are appreciable on stimulation of the ipsilateral saphenous nerve, *b*.

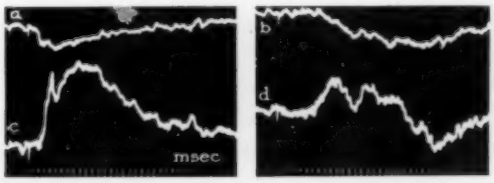

FIG. 20. Cerebellar potentials recorded from the surface and from within the cerebellar cortex on direct stimulation of Flechsig's tract, *a* and *c*, and in response to stimulation of the ipsilateral tibial nerve, *b* and *d*. The upper record of each set is of the potential from the surface.

recovery from early asphyxia, the late potentials return (*c*), but if the asphyxiation is continued farther, the first potential is also abolished at a time when activity still persists in the dorsal columns.

A stricter localization of the early potential of the cerebellar reponse to impulses afferent in Flechsig's tract is made possible by the second type of experiment (Fig. 18). By transecting the ipsilateral dorsal spino-cerebellar tract, the normal cerebellar response to stimulation of the tibial nerve, *a*, is modified, *b*, in that the early potential disappears and that the second component is greatly reduced. Both these components are still present in the response of the opposite cerebellar cortex to its ipsilateral afferent stimulation, *c*.

Since the second component of the cerebellar response is either greatly reduced or abolished when Flechsig's tract is sectioned, this potential must represent the activity of cerebellar units which are in large proportion stimulated by impulses afferent in the tract. On the other hand, the persistence

under the same conditions of the late component indicates that the cerebellar elements which cause it are activated independently of impulses from the dorsal spino-cerebellar tract.

On this point additional evidence comes from a comparison of the cerebellar responses obtained on stimulating the ipsilateral tibial and saphenous nerves (Fig. 19). The earlier potentials of the response to tibial stimulation (*a*) are absent in the activity set up by stimulation of the saphenous nerve (*b*).

Cerebellar potentials in response to direct stimulation of Flechsig's tract. A large, relatively undispersed volley of activity arrives at the cerebellar cortex when the tract is stimulated directly. Stimulation of a peripheral nerve, on the other hand, produces a more dispersed volley in the tract. This is prolonged by the repetitive activation of Clarke's cells and accompanied, during the late activity, by impulses which arrive via other pathways. Therefore, differences which appear in the cerebellar responses set up by the two types of afferent volleys throw further light on the cerebellar effects of activity in Flechsig's tract.

As recorded from the surface of the cerebellum, the electrical response is positive (Fig. 20*a*), but short in comparison with the potentials evolved on stimulating the tibial nerve, *b*. The difference appears to be due mainly to the absence in *a* of the late components of the cerebellar response observed when the tibial nerve is stimulated. The positivity recorded in *a* becomes converted into a large negative potential when the tip of the needle electrode penetrates the surface of the cortex, *c*. At the same locus the response to tibial stimulation, *d*, shows an early negativity and a large later positive potential. This positivity has the time relation of the third component of the surface potential in *b*. Its absence from the response to direct stimulation of the tract furnishes more proof that the cerebellar units which produce this potential are activated independently of impulses from Flechsig's tract.

These observations are in agreement with the experiments of Ferraro and Barrera (4), who found considerable neurological disturbances in the macaque monkey following sectioning of the dorsal spino-cerebellar tract. The disturbances became more severe when other tracts afferent to the cerebellum were also destroyed at the same time.

Summary

By following the electrical responses set up in the dorsal spino-cerebellar tract of the cat, information has been obtained on the anatomy and physiology of the tract, on the synaptology of their cells of origin, presumably lying in Clarke's column, and on the activity which is initiated within the cerebellar cortex by impulses afferent to it in Flechsig's tract. Activity in the tract has been caused either by stimulation of the afferent fibers of peripheral nerves of the hind limb or by direct stimulation of the tract.

Flechsig's tract extends from the fourth lumbar level of the spinal cord into the vermis of the cerebellum. It is set into activity by impulses in the

primary neurons of ipsilateral nerves which are afferent for muscle proprioceptors.

Impulses are conducted in Flechsig's tract nearly twice as rapidly as in the fasciculus gracilis. The greater conduction velocities in the tract are correlated with the presence of larger fibers.

Activity arises in Flechsig's tract after a delay of 0.5 to 0.9 msec. following the arrival of the primary impulses in the collaterals to Clarke's column. As a consequence of the greater conduction velocity in the tract, however, the delayed impulses overtake and pass the impulses which ascend in the fasciculus gracilis. They arrive at the medulla more than one millisecond earlier than the latter.

The responses of fibers of the tract are spikes lasting about 0.5 msec. To a single afferent volley, the response is often a series of such spikes. The fibers are able to respond again within 2.0 msec. after the beginning of an earlier response. The response evoked on stimulation of one afferent pathway frequently is conditioned by activity resulting from stimulation of another afferent nerve. The conditioning effect may take the form of inhibition, or the afferent impulses may sum their excitatory effects. The electrical data indicate that the cells of origin of the fibers of the tract receive collaterals from more than one primary sensory neuron, and evidence also points to their activation by internuncial chains of varying degrees of complexity.

In the cerebellar cortex, the fibers of Flechsig's tract are distributed to the lobulus centralis and to the adjacent folia of the culmen, principally on the same side, but with an overlap across the midline to the opposite side.

Impulses which reach the cerebellar cortex via fibers of Flechsig's tract initiate considerable electrical activity in the cerebellum. Early components of the cerebellar response to all the afferent systems activated by a volley to the tibial or peroneal nerve have been identified as largely, or entirely due to the mediation of impulses from Flechsig's tract. Another component has been shown to be produced independently of activity introduced via Flechsig's tract.

REFERENCES

1. Beck, G. M. The cerebellar terminations of the spino-cerebellar fibres of the lower lumbar and sacral segments of the cat. *Brain*, 1927, *50:* 60–98.
2. Cajal, S. Ramón y. *Histologie du système nerveux de l'homme et des vertébrés.* Paris, Maloine, 1909, 2 vol. (especially Vol. 1, pp. 323–325).
3. Dow, R. S. Cerebellar action potentials in response to stimulation of various afferent nerves. *J. Neurophysiol.*, 1939, *2:* 543–555.
4. Ferraro, A., and Barrera, S. E. The effects of lesions of the dorsal spinocerebellar tract and corpus restiforme in the macacus rhesus monkey. *Brain*, 1935, *58:* 174–202.
5. Flechsig, P. *Die Leitungsbahnen im Gehirn und Rückenmark des Menschen.* Leipzig, Engelmann, 1876, 382 pp.
6. Gasser, H. S., and Grundfest, H. Axon diameters in relation to the spike dimension and the conduction velocity in mammalian A fibers. *Amer. J. Physiol.*, 1939, *127:* 393–414.
7. Häggqvist, G. Analyse der Faserverteilung in einem Rückenmarkquerschnitt (Th. III). *Z. mikr.-anat. Forsch.*, 1936, *39:* 1–34.
8. Hursh, J. B. Conduction velocity and diameter of nerve fibers. *Amer. J. Physiol.*, 1939, *127:* 131–139.

9. INGVAR, S. Zur Phylo- und Ontogenese des Kleinhirns. *Folia neurobiol.*, 1918, *11:* 205–495.
10. KAPPER, C. U. ARIËNS, HUBER, G. C., and CROSBY, E. C. *The comparative anatomy of the nervous system of vertebrates, including man.* New York, Macmillan, 1933 (see Vol. 1, Ch. VII).
11. LARSELL, O. The cerebellum. *Arch. Neurol. Psychiat., Chicago*, 1937, *38:* 580–607.
12. MACNALTY, A. S., and HORSLEY, V. On the cervical spino-bulbar and spino-cerebellar tracts and on the question of topographical representation in the cerebellum. *Brain*, 1909, *32:* 237–255.
13. MOTT, F. W. Microscopical examination of Clarke's column in man, the monkey, and the dog. *J. Anat., Lond.*, 1888, *22:* 479–495.
14. MOTT, F. W. The bi-polar cells of the spinal cord and their connections. *Brain*, 1890, *13:* 433–448.
15. PASS, I. J. Anatomic and functional relationships of the nucleus dorsalis (Clarke's column), and of the dorsal spino-cerebellar tract. *Arch. Neurol. Psychiat., Chicago*, 1933, *30:* 1025–1045.
16. SCHÄFER, E. A. Some results of partial transverse section of the spinal cord. *J. Physiol.*, 1899, *24:* xxii–xxiv.
17. SHERRINGTON, C. S., and LASLETT, E. E. Observation on some spinal reflexes and the interconnection of spinal segments. *J. Physiol.*, 1903, *29:* 58–96.
18. SHERRINGTON, C. S., and LASLETT, E. E. Remarks on the dorsal spinal-cerebellar tract. *J. Physiol.*, 1903, *29:* 188–194.
19. STRONG, O. S. Some observations on the course of the fibers from Clarke's column in the normal human spinal cord. *Bull. neurol. Inst. N. Y.*, 1936, *5:* 378–386.
20. THERMAN, P. O. Transmission of impulses through the Burdach nucleus. *J. Neurophysiol.*, 1941, *4:* 153–166.
21. TOENNIES, J. F. Reflex discharge from the spinal cord over the dorsal roots. *J. Neurophysiol.*, 1938, *1:* 378–390.
22. VAN GEHUCHTEN, A. Etat des réflexes et anatomie pathologique de la moelle lombo-sacrée dans les cas de paraplégie flasque due à une lésion de la moelle cervico-dorsale. *J. Neurol., Brux.*, 1898, *3:* 233–252.
23. VAN GEHUCHTEN, A. *Anatomie du système nerveux de l'homme.* 3d ed. Louvain, Impr. des Trois Rois, 1900, Vol. 1, 527 pp.

INTERFERENCE FACTORS IN DELAYED RESPONSE IN MONKEYS AFTER REMOVAL OF FRONTAL LOBES*

ROBERT B. MALMO[1]

From the Laboratories of Primate Biology, Yale University, New Haven, Conn.

(Received for publication March 23, 1942)

DURING THE PAST DECADE a number of investigations by Jacobsen and his coworkers have employed various tests to study the effect on behavior of complete bilateral destruction of the frontal association areas in primates. These methods have included delayed response tests, delayed alternation tests, stick problems, peg problems, puzzle boxes, and tests of discrimination of visual pattern, brightness, size, and temporal intervals.

The delayed response problem has shown the clearest change in performance after operation. The procedure involved three successive parts: (i) cue presentation, (ii) delay interval, and (iii) the time of choice. The animal watched the experimenter put food under one of two inverted cups. At the end of the delay interval the animal was permitted to raise one of the cups and receive food if it chose the baited cup. With normal monkeys delays longer than 90 seconds were obtained; operated monkeys were unable to perform successfully with delays as short as 5 seconds. Jacobsen states (11, p. 43) that this loss of capacity for delayed response is total and permanent.

On the other hand, the operated animals were successful in all the tests of discrimination learning. In discrimination learning the animal made a choice between two stimuli which remained present throughout any one trial. The animal pulled a drawer underneath one of the stimulus objects, and choice of the positive stimulus object was rewarded with food. Jacobsen (10) found that two of three operated monkeys showed good retention of discrimination habits learned preoperatively (while the other animal showed impairment, but not complete loss). He states that there was no reduction in the ability of the animals to acquire new discrimination habits. Finan's experiments (4) have shown that temporal discrimination is still possible after operation.

Extensive ablations in other cortical regions caused no deterioration of the ability for delayed response. This was shown for the *temporal* lobes by Jacobsen and Elder (12), for the *parietal* lobes by Jacobsen (11), for the *postcentral convolutions* by Breslaw, Barrera, and Warden (1) and for *motor* and *premotor* areas by Jacobsen and Haslerud (13). To this extent it has been

* This experiment was aided by a grant from the George H. Knight Memorial Fund of the Yale University School of Medicine to Dr. Donald G. Marquis. The writer is indebted to Dr. Marquis and to Dr. Henry W. Nissen for counsel throughout the investigation and for critical reading of the manuscript. Thanks are extended to Professor Robert M. Yerkes who has given continuous support and helpful suggestions.

[1] Now Psychologist, Norwich State Hospital, Norwich, Conn.

shown that the defect in delayed response performance following removal of the frontal association areas is specific to damage of those areas alone.

Jacobsen suggested the hypothesis that the frontal lobes were essential for immediate memory. He suggested, moreover, that there may be a real dichotomy between phenomena of memory span and of learning by repeated trials—a dichotomy in the sense that they are two discrete processes, mediated through different neurological mechanisms.

Nissen and his coworkers have emphasized the learning aspect of delayed response and have suggested (18, p. 382) that delayed response is impossible for operated animals because they fail to form associations during the pre-delay period. They pointed out that the delayed response situation is a series of learning-retention trials: learning takes place at the time food is shown in one cup; retention is tested at the end of the delay interval. The operations which define delayed response (or one-trial learning) are that the learning must occur in one trial without differentially administered rewards or punishments. Their hypothesis is that only animals with intact frontal lobes are capable of one-trial learning.

This analysis is, however, just as applicable to the retention as to the learning aspect of delayed response. Their definition implies two differences between delayed response and discrimination learning: (i) single vs. repeated trials, and (ii) secondary vs. primary reward. In the pre-delay learning, the *sight* of food is a *secondary* reward because it is a stimulus which derives its reinforcing value from prior conditioning in which it has been associated with a primary reward (7, p. 350). Delayed response, on both counts, is the more fragile habit. It would be in line with the interference theory of forgetting to suppose that performance on delayed response would be more deleteriously affected by activities occurring between learning and recall than would performance on discrimination learning which is a less fragile habit.

In Jacobsen's experiments the degree of *extraneous* visual stimulation was uncontrolled. Response to such extraneous stimuli may have led, therefore, to activity which interfered with the retention of the more fragile habit (delayed response). The present experiment is designed to control the degree of visual stimulation occurring during the delay interval of delayed response in order to determine (i) whether elimination of visual stimulation during the delay period will lead to high scores on delayed response by operated animals and, if so, (ii) whether interpolated visual stimulation will affect the performance adversely. In the present experiment the indirect method of delayed response was used: light stimuli instead of food stimuli served as cues.

APPARATUS

Subjects. The animal subjects used in this investigation were one female rhesus monkey (*Macaca mulatta*) and one male mangabey monkey (*Cercocebus torquatus*). Details of age, adaption to experimental procedures, etc., are presented in the protocols for individual subjects.

Apparatus. A dark room (dimensions: 2.9 $\times$3.3 meters) was used for these experiments; a dim light on a desk served for record-taking. The only lights visible to the animals were the stimulus lights; between trials they were in darkness.

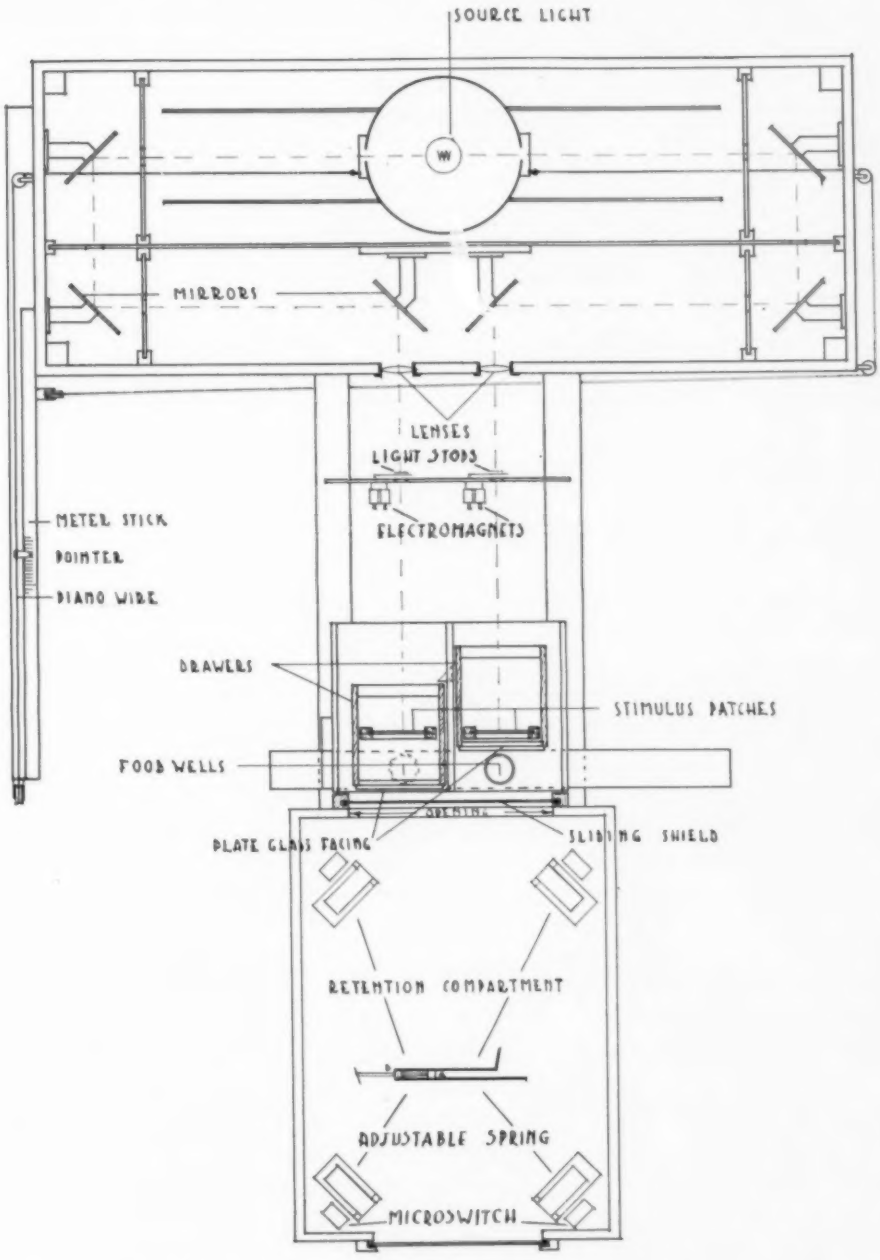

FIG. 1. Floor plan of experimental cage. The adjustable springs which were used in the activity recording apparatus are shown in the insets.

The subject was placed in a metal retention compartment (55 × 75 × 52 cm.). At the front was an opening (Fig. 1) through which it could manipulate the choice mechanism. A sliding wire-mesh screen dropped over this opening in order to prevent the animal from manipulating the choice mechanism until the conclusion of the delay period. The animal

observed the cue stimulus through the screen and through the plate glass facing (15.6 × 12.5 cm.) of a drawer against which it pushed in order to expose a food well. Pushing of one drawer automatically locked the other, so that in case a subject made a wrong response it was prevented from obtaining food immediately after that response.

The stimulus patches (2.5 cm. flashed opal glass squares, 13 cm. apart) were illuminated from behind. Light was reflected from a central source (Westinghouse T-20 1000-watt air-way beacon lamp) to each by a mirror system and lens. The brightness of the stimulus patches as measured with a Macbeth illuminometer was 3.7 millilamberts. Overheating from the lamp was prevented by a current of air generated by a fan whose sound provided a screen for the partial elimination of distracting noises, such as occasional voices from the hallway near by.

At the focal point of each lens the light passed through a small aperture, where it could be conveniently blocked to eliminate the stimulus cues during the delay interval. Blocking was accomplished by a small piece of blackened paper attached to a metal rod. This rod was fastened to the movable coil of a player piano electromagnetic unit. When the coil was energized, it rotated to the horizontal position between the pole pieces of the magnet, pulling the rod upright; when the rod was in such a position the blackened paper was removed from the focal point so that the light was no longer blocked.

Timing was regulated by the experimenter who closed a knife switch to illuminate one of the stimulus patches for approximately 2 seconds.

For presenting interpolated stimuli during the delay period, an overhead light was placed near the center of the ceiling of the retention compartment. The wire-mesh ceiling was overlaid with ground glass. A 50-watt light with metal reflector was suspended above the ground glass, and a piece of milk glass (32 × 26 cm.) was placed immediately below the bulb. A double layer of tissue paper was laid over the glass to increase the diffusion of the light. In order to provide for various intensities of overhead light a General Radiotype 100K Variac transformer was placed in circuit with the light.[2] Snap action microswitches on the control board were used to turn on the light overhead when desired. A click which was barely audible above the sound screen occurred when the switch turned on or off.

Activity recording apparatus. A graphic record of the animal's general activity in the retention compartment was obtained by means of a spring platform recorder. A fiber-board floor covered with galvanized iron was supported at the center by a ball-bearing pivot and in each corner by springs. The type of spring used is shown in the inset and the placement of these springs is shown in the floor plan of Fig. 1. The degree of tension in the spring was variable depending on the distance of block A from block B (see inset). Brass rods attached to blocks A were led outside the cage for the purpose of changing the tension on the springs. Each rod was calibrated, and a stationary pointer mounted alongside the calibrated portion served to indicate the relative degree of tension in the spring.

Beside each spring except the right front a microswitch was placed so that the trigger piece was against the under side of the top floor. The makepoles of each microswitch were wired to a signal marker.

The registration device consisted of a wax paper polygraph. In addition to the three markers for the recording of activity, a fourth marker was activated by a microswitch on the control table to indicate the beginning and end of each trial. The polygraph rollers were driven by a 4 R.P.M. Telechron motor.

In order to keep the activity apparatus approximately equal in sensitivity from day to day, the tension on the springs was regulated so that a given weight at a certain distance from each corner of the cage would close the microswitch.

Comparison of the activity records with the notes of another investigator who observed an animal through a one-way vision screen during trials on the condition of light interpolation served to establish the validity of the apparatus for the purposes of this experiment.

Procedure

After the animals had learned to push against the drawers to obtain food rewards, they were trained in a simple discrimination between light and no

[2] Macbeth illuminometer readings were taken inside the cage at the brightest part of the ground glass ceiling.

light. When an animal had made at least 10 successive correct responses on this condition, a so-called zero interval delay was given; that is, the cue light was turned on for 2 sec., turned off, and the screen was raised immediately. Short delay intervals then followed, and these were progressively lengthened until the animal was performing successfully with intervals of 10 sec. The formal experimental procedure was then instituted.

Condition 1: Non-interpolation. The stimulus light was turned on for 2 sec., the animal was retained in darkness for 10 sec., after which interval the screen was raised permitting choice of right or left by the animal.[3]

Table 1. Pre- and postoperative scores for the criterion trials with the conditions of interpolation and non-interpolation

Subject	Preoperative scores		Postoperative scores	
	Light interpolation	Non-interpolation	Light interpolation	Non-interpolation
	per cent	per cent	per cent	per cent
Mangabey no. 1	83	96	50	81
Macaque no. 7	—	—	46	94

Condition 2: Interpolation of light overhead during delay interval. The stimulus light was on for 2 sec. the animal was retained in darkness for 2.5 sec., the overhead light was turned on for 5 sec., the animal was retained in darkness for 2.5 sec. more, after which interval (10 sec. in all) the screen was raised permitting choice of right or left by the animal.

The criterion which had to be met before training was terminated was a non-improvement one. When 48 trials on each condition had been completed, performance on the first block of 24 trials was compared with that on the second. Testing was discontinued at this time only if the percentage of correct choices on the second was no higher than that on the first block of trials. In the event of improvement in either condition 24 more trials in each condition were given and the second block of trials was compared with the third. Training was continued until the percentage of correct choices on the last was no higher than that on the next-to-last block of trials for each condition. The last 48 training trials which satisfied the criterion are called the criterion trials, and all of these are presented in Table 1.[4]

The daily session was divided into six-trial blocks: interpolation and non-interpolation conditions were given in alternate blocks. Therefore any day-

[3] At the beginning of every session 5 or 6 trials with minimal delay intervals were given; that is, raising of the screen followed immediately after the disappearance of the cue stimulus. These trials were then followed by 3 or 4 more trials whose delay intervals were increased progressively up to the duration to be used in the rest of the session.

[4] Unless a score was higher than 50 per cent it was not considered as improvement even though it was greater than a score of less than 50 per cent made on the preceding 24 trials. And if the score on the first block of 24 trials was 90 per cent or greater, a higher score on the second block of 24 trials was not considered as improvement.

to-day changes in the animal's level of performance affected both conditions equally.

For the presentation of right and left choices a random order left-right sequence was used. This was designed so that an animal with a position habit or simple alternation habit could not score higher than 50 per cent on either experimental condition.

Between trials the monkey remained in darkness for a interval of from 15 to 30 sec. A daily session usually consisted of 24 trials.

Results

Delayed response under standard conditions. Table 1 summarizes the results obtained from the procedure described above. The preoperative score

Table 2. Scores for blocks of trials divided according to the condition of light interpolation and presence or absence of movement during trials (macaque no. 7)

Condition	Brightness of overhead light in millilamberts	Trials with movement			Trials without movement		Reliability of the difference	
		Per cent correct	No. trials with any movement	No. trials with movement to back of cage	Per cent correct	No. trials	S.D.	C.R.
1	0	80	65	28	97	202	.052	3.3
2	.02	73	33	3	91	96	.083	2.2
3	.07–5	63	67	1	82	178	.065	2.9
4	10	25	4	1	48	44	—	—

	Trials with movement	Trials without movement
S.D. diff. between conditions 1 and 3	.077	.031
C.R.	2.2	4.8

of mangabey no. 1 on the light interpolation condition was 83 per cent for the criterion trials. Its performance on the non-interpolation condition was 96 per cent. Both scores were reliably different from 50 per cent (the score which would have been obtained if the animal had been choosing on a chance basis). Quite different results were obtained from this animal following removal of the frontal lobes. The score on the interpolation condition was exactly 50 per cent while that on the non-interpolation condition was 81 per cent for the criterion trials.[5]

These postoperative results were in close agreement with those obtained

[5] For the two sessions in which the first 6 trials were without light interpolation (and the animal began the session with good performance) the score for all 24 trials in darkness was 96 per cent. This is precisely the score which was obtained preoperatively under this condition. On the other hand, beginning the session with poor performance (produced by light interpolations during the first 6 trials) seemed to make performance generally worse during that session.

from macaque no. 7. Its scores on the interpolation and non-interpolation conditions were 46 per cent and 94 per cent respectively.

Effect of activity during the delay period. An analysis of the activity records secured during the postoperative training and testing of macaque no. 7 reveals that although a condition of quiescence is optimal for performance in delayed response, the poorer performance obtained under the conditions of light interpolation cannot be explained in terms of increased activity due to the overhead light stimulus.

Table 2 shows the percentage of correct responses for blocks of trials divided according to the condition of light interpolation and presence or absence of movement during the trials. Absence of movement is defined as absence of any signal marker registration during the delay interval. The table shows that in every case the percentage of correct responses was higher for the non-movement than for the movement trials. It is very important to note, however, that of the 48 trials with the standard conditions of *bright* light interpolation, 44 were non-movement trials. Under this condition of light interpolation the animal failed to score higher than 50 per cent even though it did not move enough during the delay intervals to close any of the microswitches. Total activity during and between trials registered 56 times at the front and 80 times at the back of the cage during the 48 trials, showing that the sensitivity of the apparatus was sufficiently high to record movement when it occurred. On the other hand, a performance very reliably above a chance score (80 per cent in 65 trials) was obtained in darkness trials on which movement occurred during the delay interval. A further indication that the effect of light interpolation in lowering accuracy of performance was due to factors other than increase in activity comes from the fact that the score on non-movement trials without light interpolation was reliably higher than the score on non-movement trials with moderately bright light interpolation.

Almost all the animal's activity during trials was confined to the front of the cage. The number of trials in which movement to the back of the cage occurred is given for each condition in column 5 of Table 2. From these data, there is no indication that the range of activity during trials was increased by light interpolation.

It is strongly indicated that successful performance in delayed response was possible for this operated animal even when it moved to the back of the cage during the delay interval. The score on the trials where movement to the back of the cage occurred (excluding only trials from condition of *bright* light interpolation) was 72 per cent in 32 trials.[6] The probability is only 0.01 that

[6] During these 32 trials the animal chose the right side 23 times, making only 3 errors, while it chose the left side only 9 times, making 6 errors on that side. The animal did not have a right-position habit during the sessions from which these trials with movement occurred: it chose with an accuracy of 82 per cent in 89 trials when the left side was correct. That the animal did not have a general preference for the right side during this period of testing is shown by the fact that in the sessions with bright light interpolation the animal fell into a left-position habit (*i.e.*, it made almost all its errors on the right side).

this high a score would have occurred by chance. The difference between this score and 48 per cent (the score for the 44 non-movement trials with the condition of bright light interpolation) has a high reliability. The probability is only 0.014 that this difference would have resulted by chance.

INDIVIDUAL PROTOCOLS

1. Mangabey no. 1 was a vigorous and active mature male monkey (*Cercocebus torquatus*) which had been under observation for several months before training was undertaken. Prior to coming to the laboratory it had been a pet, and was unusually friendly and well adapted to handling.

Preoperative training. From June to November, 1939, this animal was used in exploratory experiments designed to determine the capacity of a normal animal to perform in the indirect delayed response situation.

The following findings resulted from this work: (i) Adaptation to the indirect delayed response situation was much slower than was that of monkeys used by other investigators in the direct delayed response situation. (ii) After the simple delayed response habit had been established it was very difficult to train the animal to retrieve food (at the back of the retention compartment) during the delay interval without disrupting the delayed response. This habit was finally mastered with delay intervals of 5 seconds or less.

After an interval of approximately six months had elapsed, further training on delayed response was given. The data from the first three sessions are summarized in Table 3. It is important to note in this table that a breakdown did not occur when the light[7] was interpolated. Thus, after an interval of six months without training, this animal was able to perform successfully in both the non-interpolation and interpolation conditions without retraining.

Table 3. Preoperative performance of mangabey no. 1 during first three sessions following a period of six months without training

Condition	Performance
No delay	10 successive correct responses
2.5 sec. delay (non-interpolation)	10 successive correct responses
5 sec. delay (non-interpolation)	1 error followed by 10 successive correct responses
5 sec. delay (2.5 sec. light interpolation)	10 successive correct responses
10 sec. delay (standard procedure as described in section on method)	
Interpolation	86 per cent correct (in 42 trials)
Non-interpolation	89 per cent correct (in 36 trials)

Following this, ten more sessions (387 trials) were given with the standard procedure in order to find the optimal length of session for the criterion trials. On the basis of this further exploration, 24 trials per session were taken as the optimal length. Then four 24-trial sessions were given; and since the scores on the last 24 trials in each condition were no higher than the scores on the preceding 24 trials, the criterion scores were computed from these 48 trials (Table 1).

When all the trials on the standard conditions were thrown together, the score on the non-interpolation condition for this animal was 92 per cent while that on the interpolation condition was 80 per cent. This is a statistically reliable difference, being 4.1 times the standard error of the difference. Although the score on the condition of interpolation was reliably higher than a chance score, it was reliably lower than the score on the condition of non-interpolation. Thus even in the unoperated subject the interpolated light acted to reduce accuracy of performance.

[7] The brightness of the overhead light used in these tests and in the tests with the standard conditions was 21 millilamberts.

Operations. The first operation was performed July 22, 1940. Under sodium amytal anesthesia, a large left-sided bone-flap was turned down. The dura was reflected and the extirpation was carried out with the Davis-Bovie electro-surgical unit by first coagulating the veins leading to the sinus. The arcuate sulcus was then incised, and the tissue lying anterior to the arcuate sulcus and its arbitrary extension to the midline was removed.

The second operation, August 5, 1940, was performed on the right side in the same manner.[8]

Postoperative tests. The first postoperative test was undertaken on September 20, 1940. The standard procedure was given, beginning with a block of 6 trials on the non-interpolation condition. Data on the postoperative retention of the habit were obscured by the presence of a strong right position preference. The subject chose the right side six times (3 errors). On the next block of 6 trials (interpolation condition) the position habit was not present. The animal chose the left side three times and made no errors. The position habit reappeared during the next 6 trials on the first condition. On the final block of trials (interpolation condition) the subject persisted in choosing the right side twelve times in succession when the left side was correct. One earlier left-side baiting in this block of trials had been responded to correctly.

Following this test session a number of sessions (totaling approximately 900 trials) were devoted to breaking the position habit and establishing satisfactory performance with a 10-second interval of delay without light interpolation. In the last of these sessions the animal's score was 95.5 per cent in 22 trials. Two trials on the light interpolation condition were also given; these revealed a strong right preference. Through this training period (as before) the correction method was used.[9] That is, when an error occurred the cue stimulus was presented again on the same side and was repeatedly presented on this side until the animal made the correct response. The error and the correction responses were scored together as one trial and one error. During the criterion trials, however, only two correction presentations were given before proceeding to the next trial. This practice was adopted in order to avoid frustrating the animal by a long series of failures in succession and to avoid greatly prolonging the session of 24 trials. The results of the criterion trials are presented in Table 1. A very strong left-sided position preference was present on the "interpolation" trials.

2. Macaque no. 7 was an immature female monkey (*Macaca mulatta*) which had been trained on brightness discrimination by another investigator before it was used in these experiments.

Preoperative training. From December, 1936, to March, 1937, the animal was trained on brightness discrimination. A brightness difference threshold was obtained. Between September and November, 1937, the brightness difference threshold was redetermined. This animal had no training on delayed response prior to operation.

First operation. A one-stage bilateral removal of the rostral part of the frontal association areas was carried out November 15, 1937. The ablation on the left side included the rostral tip of the frontal lobe and all the lateral border as viewed from above. This included the rostral portion of the Brodmann areas 9 and all of 10 and 11 and part of 12. The ablation on the right side was somewhat larger than that on the left. This ablation removed more of area 9 rostral to area 6 than that on the left side. All the frontal tip was removed, but some of the tissue above the olfactory lobe at the base of the skull still remained. All of the lateral portion of the frontal association areas back to area 8 was cleanly extirpated.

First postoperative tests. Between October and December, 1937, the postoperative brightness discrimination difference threshold was determined. The habit was perfectly retained[10] and the difference threshold had not been raised by the operation.

During the period between November, 1938, and August, 1939, a number of exploratory experiments were conducted in order to determine the capacity of this animal for performing successfully in delayed response by the indirect method under different conditions of "extra" stimulus interpolation during the delay interval.

The results of this preliminary work showed that this animal with extensive bilateral

---

[8] Both operations were performed by Dr. D. G. Marquis with the writer assisting.

[9] The correction method was not used throughout the training and testing of macaque no. 7.

[10] The score on the retention test with a brightness ratio of 1.4:1 was 80 per cent in 25 trials (beginning 9 days after the operation).

lesions in the frontal association areas could consistently make high scores on delayed response. This was true when the intervals of delay were as long as 28 seconds, providing the interior of the cage was not illuminated with a bright light. Detailed protocols for the work on this animal after the second operation removing the remaining parts of the frontal association areas are given in the next section.

Second operation—August 7, 1939.[11] "Left ablation. A left dural flap was turned back with some difficulty due to adhesions between dura and pia arachnoid. These bled very freely but were not too numerous. Ablation of the remaining tissue—all of areas 8 and 9

Table 4. Performance of macaque no. 7 under different conditions of light interpolation during period between November, 1939, and January, 1940

Session No.	Brightness of overhead light in millilamberts	Per cent correct	No. trials
	1. Whole session technique		
1	0	*94*	19
2	0.02	95	20
3	0.02	75	16
4	0	*100*	20
5	0.02	90	20
6	0.02	75	20
7	0	*95*	20
8	0.02	90	20
9	0.07	95	20
10	0.1	75	20
	2. Split-session technique		
11	0.6	52.5	20
	0	*90*	20
12	0	*90*	20
	0.6	85	20
13	0.6	80	20
	0	*100*	10
14	5	45	20
	0	*90*	20
15	0	*90*	20
	5	60	20
16	5	70	20
	0	*90*	20
	5	73	11
17	0	*100*	20
	5	85	20

—was then carried out, care being taken that no tissue was left on the base of the skull or in the frontal pole. The dura was then closed on this side, and that of the right side opened.

"Right ablation. The dural flap on the right side was not as extensive as that on the left but was sufficient to expose the desired region. On the right side the previous ablation was somewhat larger than that on the left. The present ablation comprised all of area 8 and the remaining tab of area 9 along the midline rostral to area 6."

Second postoperative training. Following the second operation, training was begun September 8, 1939. From the first the animal reacted with a strong right-sided position preference. Thirty-eight sessions were devoted to breaking this position habit and to finding suitable conditions for testing.

[11] The writer is grateful to Dr. M. A. Kennard who performed the operation and was helpful in other respects. These notes are taken from Dr. Kennard's protocols.

During the period between November, 1939, and January, 1940, tests were carried out to determine the effect on performance of interpolation of light from overhead during the interval of delay. The intra-trial conditions were the same as those described in the section on method. After several sessions without light interpolation, ten whole sessions were devoted to either the "interpolation" or "non-interpolation" condition. These sessions were followed by seven sessions where the first 20 trials were with one condition and the last 20 trials with the other.

The results of these sessions are shown in Table 4. Two points should be especially noted: (i) Performance in darkness was consistently high throughout (90 per cent correct, or better) regardless of the just previous performance on the condition of light interpolation. (ii) Performance as high as 85 per cent correct was obtained with the condition of light interpolation when the brightness of the overhead light was as great as 5 millilamberts.

In January, 1940, the standard conditions described in the section on method were used. That is, six trials on one condition were followed by six trials on the other, etc. The brightness of overhead light was 10 millilamberts throughout these sessions. From these sessions the criterion trials reported in Table 1 were taken. A strong right-sided position preference was present on the "interpolation" trials.

Immediately after the completion of the standard tests the same procedure was followed in two more sessions during which the brightness of the interpolated light was reduced to 0.1 millilambert. Thirty-six trials were given on each condition. The scores were 89 and 97 per cent for the conditions of light interpolation and non-interpolation respectively.

Discussion

The results of the present experiment may be summarized briefly as follows: successful performance in delayed response is possible for monkeys after their frontal association areas have been removed bilaterally. The difference between normal and operated monkeys with respect to such performance is not one of presence or absence of the capacity for delayed response,[12] but rather the difference is one of degree of susceptibility to the interfering effects of extraneous stimuli occurring during the delay interval.

Jacobsen's hypothesis that immediate memory is functionally localized in the frontal lobes is not sufficient to account for the results of the present experiment. These results indicate, rather, that there is no real dichotomy between phenomena of memory span and of learning by repeated trials; that is, no dichotomy in the sense that they are two discrete processes, mediated through different neurological mechanisms. In the absence of any such dichotomy the problem becomes one of relating the neurophysiological data concerned with forgetting to present theories of forgetting. The delayed response procedure is very similar to that used in studying retroactive inhibition. The following hypothesis is therefore tentatively advanced: removal of the frontal association areas of the cerebral cortex renders an animal (or human subject) more susceptible to retroactive inhibition.

The absence of a deleterious effect of extraneous stimuli on discrimination learning and retention can be accounted for in terms of the greater degree of learning occurring with a primary rather than with a secondary reward (2, p. 82). McGeoch (16) has shown that the degree of retroactive inhibition

[12] The results of the present experiment indicate that there is no difference between the effect of frontal lobectomy on the indirect as compared with the direct method of delayed response.

varies inversely with the degree of learning of the original material. The limiting case between delayed response and discrimination learning occurs in Finan's (5) experiment which showed that *one* food reward was sufficient to establish in operated animals a position habit which could be reversed by one food reward on the opposite side. One major difference between "one-trial" learning (delayed response) and "one-reward" learning (Finan's procedure) is the administration of a primary (food) reward in the latter case and the absence of any primary reward during learning in the former case. The effects of extraneous stimuli on discrimination learning may have been insufficient to produce forgetting because the degree of learning with the food reward was great enough to counteract the effects *of those particular uncontrolled interpolations.* In delayed response, on the other hand, the mere sight of food was a less strong reinforcement, and the degree of learning, for this reason, may have been insufficient to withstand the interfering effects of extraneous stimuli.

Further experimentation is necessary in order to determine the validity of this interference hypothesis. Investigations in the field of human memory have shown that the degree of retroactive inhibition is greater when the interpolated activity is similar to the activity accompanying learning than when it is dissimilar. An interpolated stimulus in the delayed response experiment might be expected to have, therefore, a greater effect if it were very similar to the cue stimulus than if it were not.

Melton (17) has suggested that susceptibility to retroactive inhibition as a function of injury to the nervous system should be studied. The results of the present experiment indicate the probable fruitfulness of studies following Melton's suggestion. This problem might be further attacked with procedures other than the delayed response; for example, discrimination learning and conditioning.[13]

Another line of investigation which would have a very direct bearing on the present hypothesis is experimentation with human subjects: the comparison of the degree of retroactive inhibition obtained from a clinical group of frontal lobe patients with that from a control group of normal subjects (and subjects with other than frontal lobe lesions) matched with the experimental group in every respect except presence of brain injury.[14]

In short, the breadth of the present hypothesis demands a broad experimental verification from several lines of study. Since retroactive inhibition is a well-established phenomenon of great importance to systematic psychology, and since it is susceptible to investigation with animal as well as with

[13] Hill (8) has demonstrated "retroactive inhibition" in conditioning.

[14] The use of "psychosurgery" (6) in treating mentally ill patients offers singular opportunity for systematic psychological research. With such patients it would be possible to compare data obtained preoperatively with that obtained postoperatively. As Hunt (p. 162) indicates, however, preoperative psychotic states (such as inattention) may obscure the capacity for memory which the patient actually possesses. But with test materials which have a high attention value, it is possible that valid preoperative data may be obtained from many patients for whom frontal lobotomy has been prescribed.

human subjects, this hypothesis holds promise as a lead to experimentation which will serve to integrate the more precise data from animal experimentation with the data from clinical studies.

Encephalization. That the cerebral cortex should have the major rôle in the mediation of such a behavioral function as memory in the primates is to be expected from the principle of encephalization. Although this principle has had its widest usefulness in the fields of motor functions (3) and sensory functions (14, 15), its application to higher mental functions may prove fruitful. The general statement of this principle is that the central nervous system shows an orderly and progressive development in the phylogenetic series, characterized by the shifting of functions from lower to higher centers and by the increasing dominance of the higher centers in the control of activity.

In the present connection the principle of encephalization would be supported if infraprimates have subcortical centers which perform the same functions as do the frontal association areas in primates. The principle of encephalization could be extended beyond purely sensory and motor functions if frontal lobe removal in the rat or dog and cat had little or no effect on delayed response performance, and if lesions in the thalamo-striate complex produced a marked impairment of memory in these lower animals.

Neuroanatomical data (19, pp. 136–138) suggest that the frontal association areas may have a way station in the thalamus (the nucleus medialis dorsalis) which is analogous to the lateral geniculate body in its relation with the striate cortex of the occipital lobes. The nucleus medialis dorsalis projects only to the frontal association areas (not to the motor and premotor areas) of the cerebral cortex; and this nucleus and the cortical area to which it projects attain their greatest development in the higher primates and man.

Carrying the analogy between the visual system and the "memory system" further, one could look for an analogue of the tectum in the thalamo-striate complex. Such a search would involve a systematic series of lesions in the thalamus and corpus striatum of infraprimate animals with accompanying behavioral experimentation along the lines of the present study.

Summary

After bilateral removal of the frontal association areas, monkeys succeeded in delayed response performance when darkness was maintained during the delay interval. Unlike normal animals, however, the operated animals failed when a bright light was turned on in the cage during the delay interval. The indirect method of delayed response was used throughout the experiment; that is, light—instead of food—was used as the cue stimulus.

These results make necessary the revision of previous hypotheses concerning the functions of the frontal association areas. The hypothesis is suggested that removal of the frontal association areas in primates leads to a marked impairment in their general capacities for memory, because the loss of these areas renders them more susceptible to retroactive inhibition.

REFERENCES

1. Breslaw, B., Barrera, S. E., and Warden, C. J. The effect of removal of the post-central convolution of the Macacus rhesus monkey upon the delayed response. *J. comp. Psychol.*, 1934, *18:* 207–226.
2. Cowles, J. T. Food-tokens as incentives for learning by chimpanzees. *Comp. Psychol. Monogr.*, 1937, *14*, no. 5, 1–96.
3. Dusser de Barenne, J. G. Some aspects of the problem of "corticalization" of function and of functional localization in the cerebral cortex. *Res. Publ. Ass. nerv. ment. Dis.*, 1934, *13:* 85–106.
4. Finan, J. L. Effects of frontal lobe lesions on temporally organized behavior in monkeys. *J. Neurophysiol.*, 1939, *2:* 208–226.
5. Finan, J. L. An analysis of frontal lobe function in monkeys by means of two "delayed response" methods. *Psychol. Bull.*, 1940, *37:* 496–497.
6. Freeman, W., and Watts, J. W. *Psychosurgery*. Springfield Ill., Charles C Thomas, 1942, xii, 337 pp.
7. Hilgard, E. R., and Marquis, D. G. *Conditioning and learning*. New York, D. Appleton-Century, 1940, xi, 429 pp.
8. Hill, C. J. Retroactive inhibition in conditioned response learning. Ph.D. dissertation, 1941, Yale University.
9. Hunt, T. Intelligence and personality profile studies, pp. 153–181 in: Freeman, W. and Watts, J. W. *Psychosurgery*. Springfield, Ill., Charles C Thomas, 1942.
10. Jacobsen, C. F. A study of cerebral function in learning. The frontal lobes. *J. comp. Neurol.*, 1931, *52:* 271–340.
11. Jacobsen, C. F. Studies of cerebral function in primates. I. The functions of the frontal association areas in monkeys. *Comp. Psychol. Monogr.*, 1936, *13*, no. 3, 1–60.
12. Jacobsen, C. F., and Elder, J. H. Studies of cerebral function in primates. II. The effect of temporal lobe lesions on delayed response in monkeys. *Comp. Psychol. Monogr.*, 1936, *13*, no. 3, 61–65.
13. Jacobsen, C. F., and Haslerud, G. M. Studies of cerebral function in primates. III. A note on the effect of motor and premotor area lesions on delayed response in monkeys. *Comp. Psychol. Monogr.*, 1936, *13*, no. 3, 66–68.
14. Marquis, D. G. Effects of removal of the visual cortex in mammals, with observations on the retention of light discrimination in dogs. *Res. Publ. Ass. nerv. ment. Dis.*, 1934, *13:* 558–592.
15. Marquis, D. G., and Hilgard, E. R. Conditioned responses to light in monkeys after removal of the occipital lobes. *Brain*, 1937, *60:* 1–12.
16. McGeoch, J. A. The influence of degree of learning upon retroactive inhibition. *Amer. J. Psychol.*, 1929, *41:* 252–262.
17. Melton, A. W. A note on the relation between brain injury and retentiveness. *Psychol. Bull.*, 1931, *28:* 175–180.
18. Nissen, H. W., Riesen, A. H., and Nowlis, V. Delayed response and discrimination learning by chimpanzees. *J. comp. Psychol.*, 1938, *26:* 361–386.
19. Walker, A. E. *The primate thalamus*. Chicago, University of Chicago Press, 1938, xxiii, 321 pp.

FURTHER STUDY ON TRANSMISSION IN AN ISOLATED NERVE-MUSCLE FIBRE PREPARATION

STEPHEN W. KUFFLER
Kanematsu Memorial Institute of Pathology, Sydney Hospital, Sydney

(Received for publication March 30, 1942)

INTRODUCTION

THE PROPERTIES of the endplate potential (e.p.p.) set up by a nerve impulse at the myoneural junction have been studied in detail in curarized muscle (3, 16). The time course of the e.p.p. in the normal cat's soleus and frog's sartorius, after the spike had been initiated, was investigated by Eccles and Kuffler (6). A study of the eserine effect on neuro-muscular transmission revealed that it was mainly due to a lengthening of the local potentials (e.p.p. +later slow wave) at the junctional region (3). Further, good evidence shows that the e.p.p. is normally responsible for the setting up of the propagating muscle impulse (18).

From all the above investigations it becomes evident that underlying the e.p.p.—or at least part of it—is the transmitter's depolarizing action at the nerve muscle junction. It was therefore important to determine in the single nerve-muscle fibre the duration and intensity of this depolarizing action, causing the e.p.p. Thus the time course of the e.p.p. is determined by two factors: (i) the "active" depolarization caused by the transmitter, whatever its nature may be, comparable in its action to an applied current pulse, (ii) the passive decay of the charge which had been built up. Duration and intensity of the 'active' period (i) was studied in normal and curarized preparations. Also, further observations of the condition of the muscle membrane during the propagating spike have been made. The effect of catelectrotonic potentials, produced by application of cathodal current pulses to the muscle fibres, has been compared with that of the e.p.p. (Section II).

METHOD

The technique of dissection, stimulation and recording has been described previously (18, 19). When constant current pulses were applied (Section II D) three non-polarizable electrodes were used, one serving as a common stimulating and recording lead. The electrodes consisted of chlorided Ag-wire dipping into a glass tube with a narrow opening and containing saline. A fine thread of cotton wool was pushed through the opening of the glass tube and made a fluid contact with the muscle fibre over a stretch of about 1 mm.

Owing to the ohmic resistance drop in the common path of the recording and stimulating circuits, part of the polarizing potential was superimposed on the electrotonic potential. The polarizing potential thus recorded rises and falls instantaneously with application or withdrawal of the current pulses and therefore can be balanced without difficulty (24).

RESULTS

SECTION I

During the numerous dissections in the course of these following and earlier experiments some further observations relating to the multiple innervation of muscle fibres have been made. Experimental evidence had already

been obtained for the existence of multiple innervation of practically all muscle fibres in the frog's sartorius (17). These tests when applied to the M. adductor longus gave similar results.

The nerve of the M. adductor longus enters generally at the pelvic end of the muscle and runs distally, giving off small branches. In a few experiments two separate nerve entries, branching from the main nerve and terminating 10–15 mm. apart on one muscle fibre, could be preserved and dissected clear. Thus it was possible to stimulate the isolated muscle fibre through its nerve at two separate points. In some of the experiments below the 'antidromic' stimulus was applied through such a second innervation on the fibre.

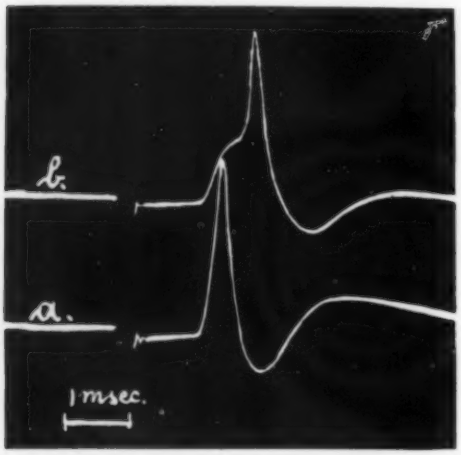

FIG. 1. Potentials recorded in the saline-paraffin interface at the same position at the region of nerve entry. *a*, strong stimulus to nerve *b*, weaker nerve stimulation. The potentials show the same difference in spike latency and in the rise of the e.p.p. component as found when the recording electrode is moved to different positions a small distance apart (18).

In one experiment two different nerve fibres terminated on the same muscle fibre within 1.0 mm. from each other. Alteration of the stimulus strength to the nerve, while recording at its entry into the muscle, revealed a step-like variation in the size of the initial e.p.p. while the latency of the muscle spike itself was altered (Fig. 1). It was suspected that two different nerve fibres of varying threshold, terminating more or less at the same region, were excited separately. This could be proved by moving the recording electrode to different positions around the nerve entry and stimulating with different stimulus strengths. The size of the e.p.p. varied according to the position (18), and also the latency of the muscle spike peak clearly indicated the existence of two separate endplate regions very close together.

In 11 single nerve-muscle preparations altogether similar tests as above were carried out, but only in one experiment (Fig. 1) was there an indication of different nerve fibres innervating closely adjoining regions on the same muscle fibre. The threshold for nerve excitation and the subsequent initiation of a propagated muscle spike remained usually constant within 1–1.5 per cent and further strengthening of the stimulus did not affect the response. It seems likely that in most cases the innervation of an endplate region is supplied by one nerve fibre.

SECTION II

A. *The e.p.p. after initiation of a muscle spike*

If a nerve impulse reaches the neuromuscular junction (n.m.j.) the e.p.p. is set up there and attains a potential as large as the subsequently initiated

muscle spike (18). This is illustrated in Fig. 2. The e.p.p. forms about 90 per cent of the rising part of the action potential, indicating that leading is practically confined to the endplate. After the diphasic trough the potential rises again and reaches 55 per cent of the initial peak potential about 2.3 msec. after the beginning of the e.p.p. The negativity after the main action potential resembles the initial e.p.p. in being largest at the endplate region and diminishing rapidly as the recording electrode is moved away from this region. This, together with other tests (below) clearly indicates that the late potential after the spike represents the further course of the e.p.p. The time course of the intervening phase of the e.p.p. is obscured by the diphasic trough. This trough and the difficulty of estimating the potential time course with interface recordings prevents the accurate estimation of the e.p.p. immediately after a spike had been set up. It can be said, however, that the muscle membrane about 2.3 msec. after the potential has started is much less readily depolarized than normally; a second nerve impulse reaching the endplate at such a time adds only about 10 per cent to the already existing potential.

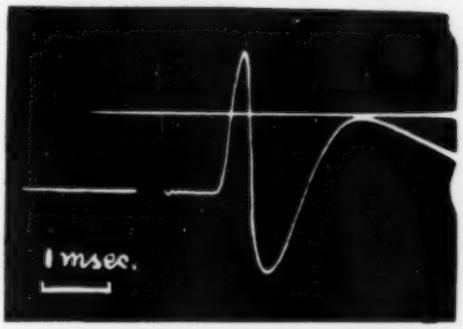

FIG. 2. Interface recording at the endplate region. The potential after the spike rises to 55 per cent total action potential height.

The time course of the e.p.p. in normal muscle can be estimated more accurately from recordings in paraffin oil (18, 19). With this method, however, the contact of the leading electrode cannot be confined to the endplate exclusively and thus the e.p.p. will be diminished relative to the spike, *i.e.* the first phase of the potential will have a greater spike component than Fig. 2. Some allowance has to be made when calculating the true time course of an e.p.p. from such a composite potential.

Two methods can be employed to estimate the time course of the e.p.p. at the endplate.

(i) The action potential set up by a nerve impulse is recorded at the endplate region and a few mm. away from it. A comparison of the two potentials will reveal the additional negativity at the endplate region which is due to the e.p.p.

(ii) Comparison with 'antidromic' muscle impulses can be made. The 'antidromic' impulse is set up by stimulation of the muscle fibre a few mm. from the nerve entry and represents a 'simple' muscle spike without an e.p.p. (19). This 'antidromic' does not differ from a spike due to indirect stimulation (*i.e.* via the nerve) if the recording is done some mm. off the endplate region (cf. Fig. 3B).

A combination of method (i) and (ii) was employed and is illustrated in Fig. 3. Thus Fig. 3A shows an action potential set up by a nerve impulse (N)

at the n.m.j. The negativity following the diphasic wave rises to about 25 per cent of the initial peak potential by 2.2. msec. after the potential start and has almost disappeared in about 7 msec.

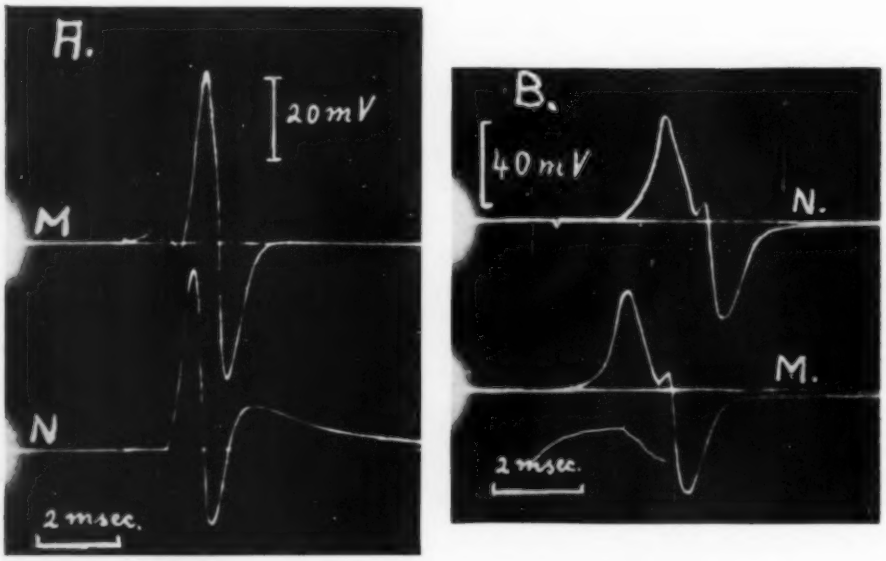

FIG. 3. *A*. Potentials recorded in paraffin at the nerve-muscle junction (n.m.j.). *N* due to nerve stimulation, *M* due to 'antidromic' stimulus. *B*. Potentials 3 mm. from the n.m.j. Note that the two potentials *N* and *M* are nearly identical. (Base line is shown in each record).

The 'antidromic' (M) Fig. 3A was recorded at the same position and no negative wave follows the diphasic phase. It is safe to assume that the main difference between the M and N responses is due to the e.p.p. set up by N.

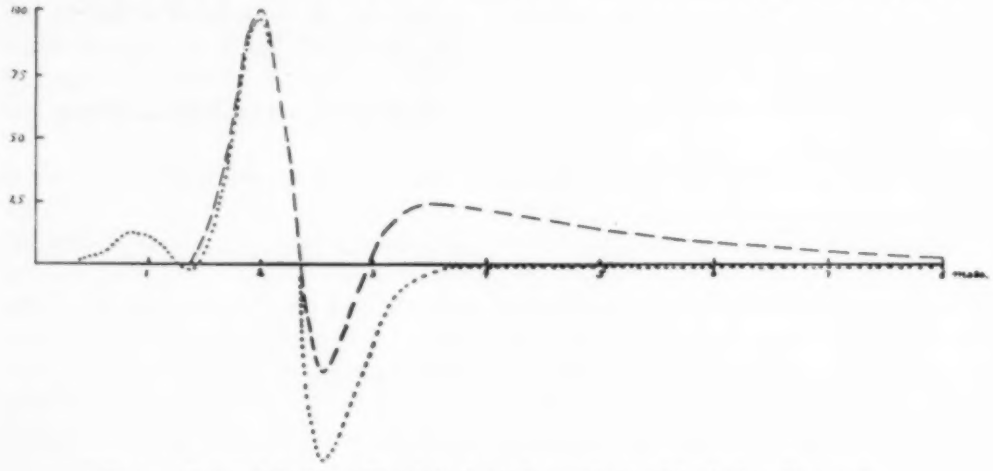

FIG. 4. Potentials from Fig. 3*A* plotted together, with their spike peaks synchronized. The difference due to e.p.p. is well seen.

Besides this, some variations between the two potentials are also expected owing to small lead artifacts which affect M while it propagates towards the endplate region, *e.g.* the small hump before the potential rise was due to a saline blob at the nerve entry. Moreover, the N response is 'growing up' and spreading outwards from the lead, while with M the fully grown impulse propagates past it.

If the M and N responses are recorded 3 mm. from the end-plate region they are practically identical (Fig. 3B). With successive movements of the recording electrode away from the end-plate to this position, all transitional diminutions can be seen in the late negativity of the N response; M on the other hand is not significantly altered.

If M and N are plotted together as in Fig. 4 so that their spike peaks are synchronized the difference between them becomes evident and gives the approximate time course of the e.p.p. following the N spike.

Thus for the normal muscle the approximate e.p.p. time course can be shown before it sets up the muscle spike, (the initial e.p.p., cf. 18) and about 1 msec. later the late part of the e.p.p. can be seen.

B. *Transmitter action as tested by interaction between an 'antidromic' spike and an impulse set up by nerve stimulation.*

The muscle fibre was stimulated some distance away from the endplate region and a muscle spike (M) was set up propagating towards the nerve-muscle junction (19). At different intervals afterwards a nerve impulse was initiated arriving at the endplate synchronously with M or at short intervals later. If it forestalled M, *i.e.* reached the junction earlier, the usual e.p.p. and the consequent muscle impulse was set up and therefore M could not be recorded. The two muscle spikes—one coming towards, the other from the endplate—collided and extinguished each other. If M reached the endplate or was very near to it when the e.p.p. was just rising, the antidromic spike was 'speeded up,' as its 'foot' summed with the developing e.p.p. (cf. also later Fig. 6a, 7b). In Fig. 5 the MN interval is so timed that the transmitting agent in setting up the e.p.p. would start at the middle of the rising phase of M (marked by arrow). The two exposures, the antidromic (M) alone (a) and then M and N together (b), have been super-

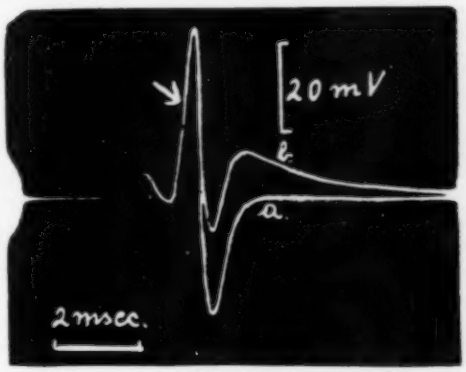

FIG. 5. Interaction between an 'antidromic' muscle impulse and nerve impulse in normal muscle recorded in paraffin. (*a*) Antidromic alone. (*b*) Antidromic + nerve impulse, both exposures superimposed. The action of the nerve impulse starts before the spike peak of *M* (arrow) but does not add a potential for about 0.6 msec. (see text).

imposed and thus the effect of N can be clearly seen as an addition. The nerve impulse arrives at the myoneural junction much too early to set up a muscle impulse after the M spike. There is not even the usual e.p.p. addition as in the early phases of the refractory period (7, 5, 6, 19). During the rising phase (after the arrow), on the spike peak and on the greater part of the falling phase no additional potential can be observed. But eventually a potential is built up, seen as addition to M (Fig. 5). Clearly the depolarizing action of the transmitter gave no recordable potential while the muscle spike (M) was passing the endplate region, and it only became observable late in the decaying phase of the spike. Some inferences can be drawn from the above observation.

(i) during the spike the muscle membrane is fully depolarized, as a nerve impulse, which by itself causes a full depolarization of the membrane, has no appreciable additional effect.

(ii) the depolarizing agent produced by the nerve impulse outlasts the spike and builds up a potential in the refractory muscle. About 2 msec. after the nerve impulse had reached (arrow) the endplate this potential starts to decay.

Thus from the above experiment it can be concluded that the transmitter action lasted *at least* 2 msec. at 28.0°C. Longer durations were observed in other experiments at similar temperatures, and at about 20.0°C. the average duration of transmitter action, calculated as above, lasted 3–4 msec. (cf. effect of temperature on e.p.p., 3). The onset of the decay of the added potential does not imply a cessation of the depolarizing agent. It will be shown in part C, that there is still some transmitter action left during a part of the decaying phase of the curarized e.p.p. A similar action can be assumed to persist after the added potential (Fig. 5) starts to diminish.

Thus the method above does not take into account the decaying part of the potential addition and therefore the full duration of transmitter action has not been derived. From the quick decay, however, it can be concluded that the intensity of the transmitter action decreases rapidly during that time.

C. *The action of curarine*

A subparalytic dose of curarine has two main effects: (i) it diminishes the e.p.p., (ii) it shortens its duration. After neuromuscular transmission has been blocked an e.p.p. alone is set up. Additional application of curarine only diminishes the e.p.p. still more, without altering its time course, *i.e.* no appreciable further shortening is observed (3, 5, 6, 19).

In the following experiments the same method as in B is applied to completely curarized muscle. The interaction between the antidromic (M) and the pure e.p.p. due to nerve stimulation (N), is illustrated in Fig. 6, 7, 8. The findings were similar to those of Eccles, Katz and Kuffler (3) but could be executed more accurately on the single fibre (cf. discussion). The records were taken with the preparation in paraffin, the curarine action being just paralytic.

If the e.p.p. rise starts synchronously with the arrival of the antidromic it disappears completely during the spike, but appears again, although diminished, after the spike had passed the n.m.j. This shows that the depolarizing action of the transmitter has not ceased by that time. In Fig. 6b the e.p.p. action begins just at the spike peak of M. No addition occurs at

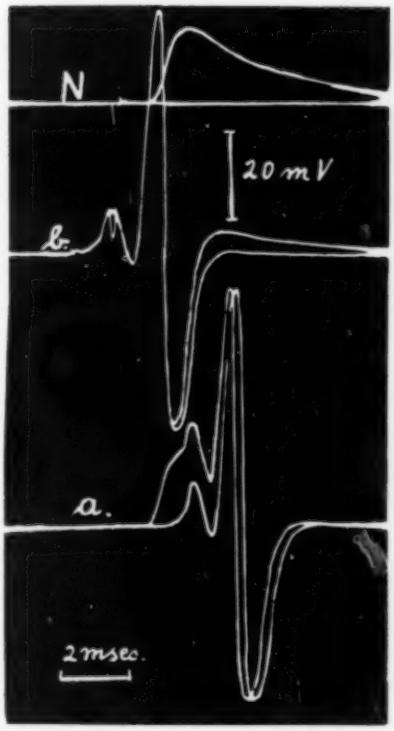

FIG. 6. Interaction between 'antidromic' muscle spike and e.p.p. in curarized muscle, recorded in paraffin at the n.m.j. The *N* stimulus in each record is fired at a constant position on the sweep, while the antidromic spike reaches the n.m.j. before (*b*) or after (*a*) the e.p.p. is set up. Top record *N;* e.p.p. due to nerve impulse; (*b*) antidromic alone and together with *N*. *N* adds only a reduced potential (as compared with *N* alone) to the later part of the spike. (*a*) the antidromic spike is 'speeded up' by the e.p.p., which 'collapses' entirely during the spike, as no potential addition is built up after the diphasic wave similar to *b*. The hump in front of the antidromic spike is due to a saline blob at the nerve entry into the muscle fibre, acting as a 'false lead.'

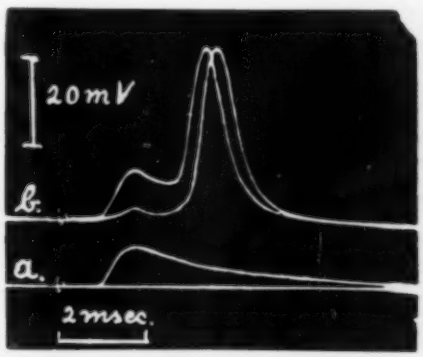

FIG. 7. Action potentials recorded in paraffin. The potential is made monophasic by injuring the fibre near to the recording lead. *a*. e.p.p. due to nerve impulse. *b*. an antidromic muscle spike alone, and then together with the e.p.p. 'Speeding' effect can be observed, e.p.p. disappears during the spike (cf. Fig. 6*a*) and is not built up again after the spike.

first and then gradually a small potential is built up, reaching about 45 per cent of the control e.p.p. (cf. +0.8 msec. in plottings of Fig. 8). The time course of this addition suggests that a fairly strong action of the transmitter still persists (in the above experiment) about 3 msec. after its start. In Fig. 6a the e.p.p. action begins 1.6 msec. before the antidromic spike (M) has reached the endplate. M is speeded up, *i.e.* although initiated at the same time as previously, reaches the recording electrode earlier (3); but no appreciable further effect on M is seen. The e.p.p. disappeared entirely during the spike of M. The depolarizing agent underlying it could not build up a

potential similar to Fig. 6b after the antidromic had passed. Evidently, its action had ceased by the time the muscle membrane had recovered sufficiently for a potential to be built up again. Thus no appreciable depolarizing action remains about 4.0 msec. after the beginning of the e.p.p.

The 'speeding' effect and the 'collapse' of part of the decaying phase of the e.p.p. is illustrated more clearly in Fig. 7. The action potential had been made monophasic by injuring the muscle fibre a short didstance from the junctional region. Such an injury spreads quickly and renders the muscle

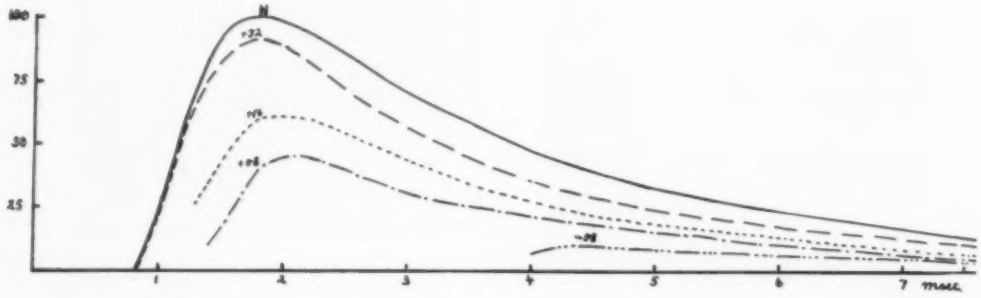

FIG. 8. Plottings of records from experiment partly illustrated in Fig. 6. E.p.p.'s. during or after passage of spike as p.c. of normal e.p.p. (*N*). E.p.p. starts +0.8, +1.6 or +3.2 msec. *after* spike has reached the endplate region. (note the recovery of e.p.p. size.) If e.p.p. set up −0.8 msec. *before* spike reaches endplate, it collapses during the spike but builds up a reduced potential 3.0–3.5 msec. after its start.

inexcitable in a few minutes. 'Speeding' in excess of 0.2–0.3 msec. has not been observed, presumably as it occurs only during the 'foot' of the spike. Only then can the subsequent breakdown be speeded up by summation of the two subthreshold potentials, *i.e.* foot of the spike and e.p.p. At times the 'speeded' spike was found a few p.c. bigger than the 'antidromic' alone. The plottings of Fig. 8 are from the experiment partly illustrated in Fig. 6, the 'antidromic' reaching the endplate region at different intervals before (+) or after (−) the e.p.p. had been set up (cf. legend).

From the above it can be concluded, that some transmitter action is still left 3.0–3.5 msec. after the e.p.p. had started, as after 'collapsing' it still builds up a diminished potential at such a time.

The method described in this section (C) allows a more accurate estimation of the duration of the transmitter action because the antidromic can be timed to arrive at the endplate also after the e.p.p. had been set up. This was impossible in uncurarized muscle (Section B), for, if the nerve impulse forestalled the antidromic (M), it set up a spike besides the e.p.p. and thus prevented M from reaching the junctional region.

The persistence of the depolarizing agent in curarized muscle is thus found to be about 4 msec.

D. *Application of constant current pulses*

It has already been pointed out (3, 13, 14, 23), that the effect of the local potential at the endplate region is similar to a catelectrotonic potential. It was seen above (B and C) that an e.p.p. applied during the spike has no effect on the greater part of its rising and falling phase. Similar findings were obtained when applying a cathodal current pulse during the spike. If a subthreshold catelectrotonic potential is set up (cf. Method) it rises in an approximately exponential way and after withdrawal of the current pulse it

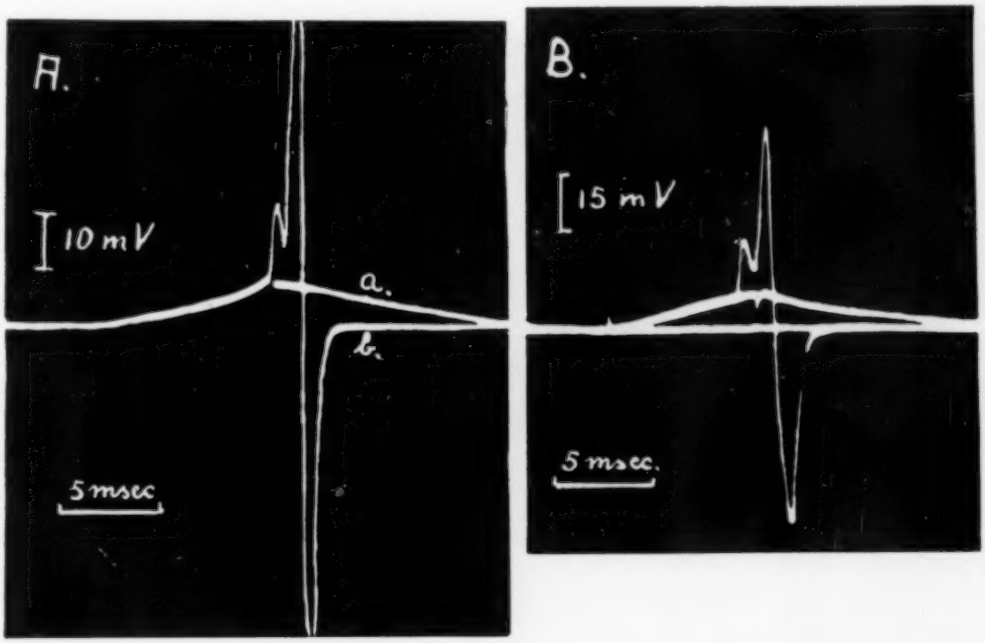

FIG. 9. Recordings in paraffin. *A:* (*a*) just subthreshold catelectrotonic potential, (*b*) the catelectrotonic potential reaches threshold and sets up a spike; the diphasic wave returns to the base line. *B:* a spike initiated some distance away from the recording electrode passes a region where a just subthreshold catelectrotonic potential has been set up. The electrotonic potential 'collapses'. (In *A* and *B* the spike if recorded alone without the catelectrotonic potential shows no difference in height or shape (see text).)

decays similarly (cf. Fig. 9B and also 1, 21, 10, 11, 24, 14, 16). In Fig. 9Aa, a just subthreshold catelectrotonic potential was applied. If the current is withdrawn 0.5 msec. later (Fig. 9Ab) a propagating response is set up (the two exposures are superimposed). After the diphasic wave the potential returns to the base line in the same way as a propagating muscle spike, set up by direct stimulation; there is no addition of potential to the spike which could be attributed to a part of the decaying catelectrotonic potential surviving during or after the spike. It has 'collapsed' during the spike in the same way as the decaying phase of the e.p.p. in Fig. 6a, 7b.

Similarly a subthreshold catelectrotonic potential is completely abolished by the passage of a spike set up by direct stimulation elsewhere on the fibre (Fig. 9B).

Note the striking difference in the ratio between just subthreshold or threshold catelectrotonic potential and spike size (Fig. 9 and 10). The reason is twofold: (i) in 9A the contact of the common stimulating-recording lead was larger than in 9B, *i.e.*, it recorded the catelectrotonic potential further away from its origin (24), (ii) the threshold value to set up a spike varies after long lasting stimulation or slight damage (11, 20).

The effect of activity diminishing the electrotonic potential set up by an applied current pulse has first been described by Bernstein. (For a full discussion of the subject see Katz, 15, chapt. IV and V.)

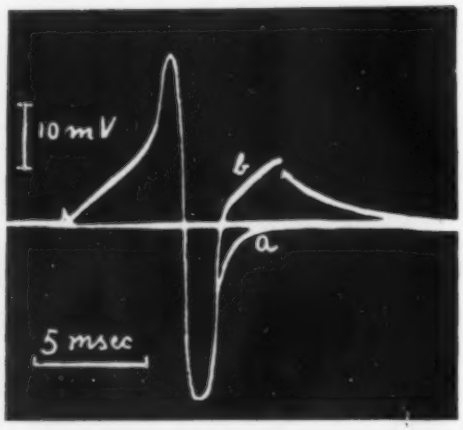

FIG. 10. *a*. The current pulse was withdrawn just when the spike was set up. *b*. The current pulse was continued and a potential is built up after the diphasic wave while the greater part of the spike is not affected. After withdrawal of the current pulse the additional potential decays approximately exponentially. Note the unbalance when current pulse discontinued, shown as an instantaneous potential shift (see text).

In Fig. 10 two exposures have been superimposed. In the first (i) the current pulse was withdrawn immediately after a spike had been initiated. In the second (ii) the current pulse was continued for several msec. The continuation of the current pulse has no effect during the spike itself, but later builds up a potential. This seems to be analogous to the continued depolarizing action of the transmitter producing the e.p.p. in Fig. 5, 6b. The time course of the potential actually built up after the spike by a prolonged current pulse could not be determined because the contraction of the muscle and the consequent changes in resistance between the recording electrodes effect an imbalance of the applied current pulse (cf. Method see p. 309).

When gradually increasing the strength of the constant current pulse there was evidence for local responses when the catelectrotonic potential was near threshold. An inflexion appeared on the catelectrotonic potential, which was not present on the anodal polarization potential when the polarity was reversed. This inflexion was similar to the phenomenon described by Rushton (22) for constant current stimulation of nerve. Owing to the large contact area (about 1 mm. wide) of the non-polarizable electrodes the effective recording occurred some distance from the origin of these local responses which consequently were much diminished in size. When Hodgkins (11) method was used, (brief condenser discharges and fine Pt wires) more clear cut evidence for non-propagating responses in the single muscle fibre was

found. Figure 11 illustrates the electric potential changes at the common stimulating electrode when the muscle was stimulated by short (60μ sec.) condenser discharges. The anodal potential changes increase proportionately to the stimulus strength and decay in an approximately exponential way. The effect at the cathode with a strength of 0.75 threshold differs little from the corresponding anodal potential. With the cathodal stimulus increased to 0.97 (just subthreshold) the potential change is larger and much more pro-

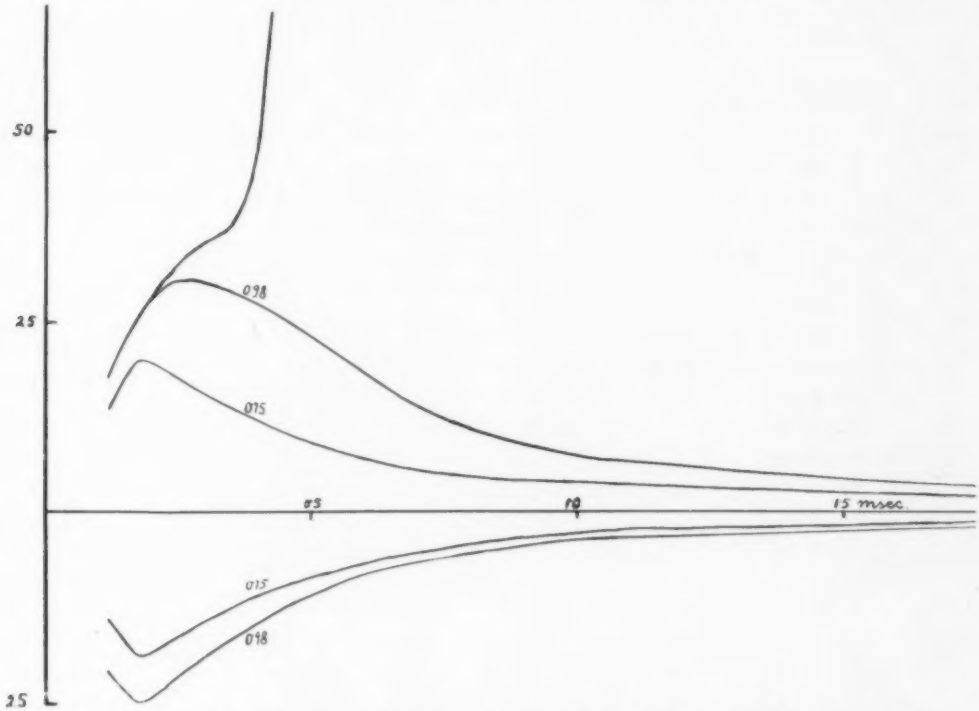

FIG. 11. Potential changes at the stimulating electrode with stimulus strength as indicated. Ordinates: potentials as p.c. of the propagated spike (75 mV).

longed than the corresponding anodal potential. With threshold (1.0) stimulation a fully grown spike of about 75 mV was set up.

From the analogy with nerve (12, 11—Fig. 7, 8, 10; 20) and also from the findings of 'abortive' impulses in refractory muscle (7, 8, 19) these results were to be expected in the normal muscle fibre.

DISCUSSION

The action of a nerve impulse on a muscle fibre may be attributed to a transmitting agent which sets up the endplate potential by actively depolarizing the junctional region of the muscle fibre. In fully curarized muscle the depolarization rises quickly to a maximum which is insufficient to initiate a spike and then slowly decays (*cf.* the pure e.p.p. time course). In curarine-free normal muscle, on the other hand, the depolarization quickly reaches

the threshold value and initiates a spike. The effect of such a depolarizing action, subthreshold or above threshold is imitated to a great extent at the cathode of a constant current pulse, *i.e.* by the catelectrotonic potential (13, 14, 23).

Some of the similarities between the e.p.p. and a catelectrotonic potential are as follows:

1. At a critical potential level a propagating spike is initiated as seen from Fig. 9 and 10 (9, 15, 11). This can be well demonstrated with the e.p.p. in curarized preparations (3, 16) and occurs also in normal muscle adjacent to the endplate (18).

2. During the greater part of the spike the application of a constant current pulse does not add a potential (Fig. 9 and 10), but if it outlasts the spike a potential is built up as the refractoriness passes off (Fig. 10b). Similarly the depolarizing action of the transmitter does not produce a potential during the muscle impulse, but only when outlasting it (Fig. 5, 6b).

3. After cessation of a current pulse the catelectrotonic potential decays passively (Fig. 9). In the e.p.p. of the curarized preparation the cessation of 'active' depolarization is gradual (Sect. II C), so only the latter part of the decaying phase is purely passive.

4. The passively decaying phase of the catelectrotonus is completely abolished by a muscle impulse (Fig. 9A and B). With the e.p.p. a spike gives a similar 'collapse' (Fig. 6a, 7b), but some potential is rebuilt when the impulse acts early in the decaying phase, thus indicating the persistence of some 'active' depolarization (cf. above and Fig. 8, −0.8 msec.).

Additional similarities have also been described (3, 6, 16, 18, 19).

5. The similarity of spatial spread of the e.p.p. and of a catelectrotonic potential are similar.

6. Both prolong the refractory period.

7. The impedance changes accompanying subthreshold current pulses and e.p.p.'s show close resemblance during their decaying phases.

From the experiments showing the building up of the e.p. p. after an antidromic spike (Sect. II B, C) it is possible to determine approximately the time course of the 'active' depolarizing action of the transmitting agent. There appears to be an intense action for 2–3 msec., becoming nearly ineffective in a further 2 msec. No conclusion can be drawn, however, concerning the nature of the transmitting agent. The duration of the depolarizing action was found to vary appreciably in different preparations.

Sections II B, C, D, clearly indicate that during a muscle impulse only the onset of the rising phase can be altered by an e.p.p. (Fig. 6a, 7b). The size and shape of the main part of the following spike potential are unchanged. If there were any readily polarizable membrane in the muscle fibre, which is not affected by an impulse, some additional potential would be added with applied current pulses (Fig. 9 and 10). Thus practically the whole polarizability of the muscle fibre must be confined to the excitable membrane which 'breaks-down' during activity (2; also earlier findings discussed by Katz, 5).

In curare-free multifibre preparations asynchronism prevented a direct investigation of the effect of an e.p.p. superimposed on the antidromic muscle spike. However, extrapolation of the later phase of such a superimposed e.p.p. suggested that the e.p.p. added about 10 per cent to the spike summit (6, pp. 490–492 and Fig. 5 and 6). But such an extrapolation has now been shown (Sect. II, B and C) to be unjustifiable.

In the curarized multifibre preparation a small addition of e.p.p. was frequently observed on the crest of the antidromic spike. This also was most likely due to asynchronism of the individual spikes, *i.e.* the observed spike peak does not coincide with every individual peak (3, pp. 373–375).

Summary

Experiments were carried out on isolated nerve-muscle fibre preparations from the M adductor longus of the frog (*Hyla aurea*).

1. The transmitter action, *i.e.*, the action of the depolarizing agent producing the endplate-potential (e.p.p.) has been further investigated in curarized and normal muscle. In normal muscle the intensity of the 'active' depolarization decreases rapidly after about 2.5 msec., and has disappeared at about 5 msec. (18–20°C.). These durations are diminished by curarine.

2. During the greater part of the rising and falling phase of a propagating muscle impulse the membrane can not be further depolarized, *i.e.*, no appreciable additional potential can be added during that period by (i) an e.p.p. (ii) a sub- or superthreshold constant current pulse. It is therefore concluded that the polarizability of the muscle membrane is abolished during activity.

3. A propagating muscle impulse approaching the endplate region is speeded up by the e.p.p. in curarized or non-curarized muscle, in agreement with earlier work on the whole muscle.

4. The effects of subthreshold and superthreshold catelectrotonic potenials, produced by constant current pulses, are shown to resemble those due. to the e.p.p. in normal and curarized muscle.

5. Local responses are set up in normal muscle when the stimulus is near threshold.

6. Some findings regarding multiple innervation of the muscle fibres in the M adductor longus are described (Sect. I).

I wish to thank Dr. J. C. Eccles and Dr. B. Katz for their valuable help and advice during the course of this investigation, and also the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

REFERENCES

1. Bogue, J. Y., and Rosenberg, H. The rate of development and spread of electrotonus. *J. Physiol.*, 1934, *82:* 353–368.
2. Cole, K. S., and Curtis H. J. Electric impedance of the squid giant axon during activity. *J. gen. Physiol.*, 1939, *22:* 649–670.
3. Eccles, J. C., Katz, B., and Kuffler, S. W. Nature of the "endplate potential" in curarized muscle. *J. Neurophysiol.*, 1941, *4:* 362–387.
4. Eccles, J. C., Katz, B., and Kuffler, S. W. The effect of eserine on neuromuscular transmission. *J. Neurophysiol.*, 1942, *5:* 211–230.

5. Eccles, J. C., and Kuffler, S. W. Initiation of muscle impulses at the neuro-muscular junction. *J. Neurophysiol.*, 1941, *4:* 402–417.
6. Eccles, J. C., and Kuffler, S. W. The endplate potential during and after the muscle spike potential. *J. Neurophysiol.*, 1941, *4:* 486–506.
7. Eccles, J. C., and O'Connor, W. J. Responses which nerve impulses evoke in mammalian striated muscles. *J. Physiol.*, 1939, *97:* 44–102.
8. Eccles, J. C., and O'Connor W. J. Abortive impulses at the neuromuscular junction. *J. Physiol.*, 1941, *100:* 318–328.
9. Erlanger, J., and Gasser, H. S. *Electrical signs of nervous activity.* Philadelphia, University of Pennsylvania Press, 1937. x, 221 pp.
10. Hill, A. V. Excitation and accommodation in nerve. *Proc. roy. Soc.*, 1937, *B119:* 305–355.
11. Hodgkin, A. L. The subthreshold potentials in a crustacean nerve fibre. *Proc. roy. Soc.*, 1938, *B126:* 87–121.
12. Katz, B. Experimental evidence for a non-conducted response of nerve to subthreshold stimulation. *Proc. roy. Soc.*, 1937, *B124:* 244–276.
13. Katz, B. The reversal of neuro-muscular block by catelectrotonus. *J. Physiol.*, 1938, *92:* 20–21P.
14. Katz, B. The 'anti-curare' action of a subthreshold catelectrotonus. *J. Physiol.*, 1939, *95:* 286–304.
15. Katz, B. *Electric excitation of nerve.* Oxford University Press, 1939*b*. 151 pp.
16. Katz, B. Impedance changes in frog's muscle associated with electrotonic and "end-plate" potentials. *J. Neurophysiol.* 1942, *5:* 169–184.
17. Katz, N. and Kuffler, S. W. Multiple motor innervation of the frog's sartorius muscle. *J. Neurophysiol.*, 1941, *4:* 209–223.
18. Kuffler, S. W. Electric potential changes at an isolated nerve-muscle junction. *J. Neurophysiol.* 1942, *5:* 18–26.
19. Kuffler, S. W. Responses during the refractory period at the myoneural junction in an isolated nerve muscle fibre preparation. *J. Neurophysiol.* 1942, *5:* 199–210.
20. Pumphrey, R. J., Schmitt, O. H., and Young, J. Correlation of local excitability with physiological response in the giant axon of the squid. *J. Physiol.*, 1940, *98:* 47–72.
21. Rushton, W. A. H. The normal presence of α and γ excitabilities in the nerve-muscle complex. *J. Physiol.* 1931, *72:* 265–287.
22. Rushton, W. A. H. A new observation on the excitation of nerve and muscle. *J. Physiol.*, 1932, *75:* 17P.
23. Schaefer, H., and Haass, P. Über einen lokalen Erregungsstrom an der motorischen Endplatte. *Pflüg. Arch. ges. Physiol.*, 1939, *242:* 364–381.
24. Schaefer, H., Schölmerich, P., and Haass, P. Der Elektrotonus und die Erregungsgesetze des Muskels. *Pflüg. Arch. ges. Physiol.*, 1938, *241:* 310–341.